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Anti-Adherent Potential of *Lavandula hybrida Grosso* Essential Oil Against *Staphylococcus saprophyticus* Strain

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ABSTRACT

The oral cavity is composed of various microorganisms that live organized in the form of biofilm that can become dysbiotic, causing oral infections. Currently, the concern of science about nosocomial infections in hospitalized patients has grown. It has been observed that *Staphylococcus saprophyticus* is a pathogen present in this type of infection. Thus, phytotherapy has gained more space because of the presence of phytochemicals with therapeutic activities. Therefore, essential oil of *Lavandula hybrida Grosso* is an option in infection therapy, due to the potential antimicrobial and anti-adherent potential against numerous microorganisms. Thus, the aim of this research was to evaluate the anti-adherent activity of *Lavandula hybrida Grosso* essential oil against SA45 strain of *Staphylococcus saprophyticus*. The assays were performed using the slant-tube technique to determine the Minimum Inhibitory Adherence Concentration (MIAC) in the presence of 5% sucrose. From this, we obtained the following results: MIAC in the value of 1:16, being lower than the potential of the Digluconate 0.12%. It was concluded then that the essential oil of *Lavandula hybrida Grosso* showed strong anti adherent potential against the strain under study.

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INTRODUCTION

The oral cavity contains a microbiota of over 1000 distinct species[1], many of these bacteria have a symbiotic relationship with the host, where in a homeostatic equilibrium [2] .However, numerous factors can unbalance this this relationship, such as poor oral hygiene, inflammatory and autoimmune diseases, carbohydrate in carbohydrates, etc., which can lead to a greater proliferation of dysbiotic microorganisms dysbiotic microorganisms [3, 4].

Both surfaces, such as the oral mucosa, as in non-flammatory surfaces such as the teeth, where the teeth, where they will form the oral biofilm, which is a collection of microorganisms that are surface and are immersed in a complex matrix of polysaccharides [5].

Hospital-acquired infections can be defined as new infections that occur 1 to 3 days after admission to the hospital, 3 days after discharge, or 30 days post surgery. These infections are caused by various microorganisms such as viruses, bacteria bacteria or fungi, and mostly occur in the bloodstream, lung, bladder, and surgical areas [6].

In chronic and critical patients in hospitals and long-stay institutions has been observed in several studies associating microbial proliferation and systemic diseases systemic diseases, since oral hygiene has great importance in health aspects. Among the most common hospital infections, aspiration pneumonia is the leading in mortality and second in most common morbidity [7, 8, 9].

Staphylococcus saprophyticus is a gram-positive, coagulase-negative coccus present in the normal human microbiota, colonizing regions such as the perineum, urethra and gastrointestinal tract[10]. It is a bacterium commonly found in the skin, gastrointestinal gastrointestinal tract and urinary tract. However, it becomes pathogenic when there is an imbalance in the imbalance of the microbiota, urinary infections appear, for example [11, 12, 13] .

It is also responsible for 10-20% of urinary infections in young women with an active sex life [14, 15, 16]. In addition, *S. saprophyticus* has raised concern due to its ability to form biofilm in the environment, which can form in hospitals, clinics instruments, among others [17, 18].

It has been observed in some studies, the resistance of *S. Saprophyticus* [31] to methicillin, which is

associated with the ability to form biofilms, as it forms a protective barrier for bacteria against antimicrobial agents, making these microorganisms highly pathogenic [13, 19, 20].

The search for treatments with natural products has been increasing more and more, since they have numerous pharmacological activities, have low cost and good popular acceptance, in addition to rare adverse effects, making it a viable option compared compared to synthetic products [21, 22]. Besides this, many researchers have been searching for alternative therapies with herbal medicines, since the irrational use of antimicrobials irrational use of antimicrobials by the population has caused resistance of microorganisms to existing synthetic drugs [22] .

Essential oils are natural products obtained from the secondary metabolism of plants, which plants, that act attracting pollinating animals, help in seed dispersal, and as a form of plant defense. as a form of defense for plants. These oils are liquid, volatile, and lipophilic, and can be found in various parts of the plant, such as flowers, leaves, roots, fruitsamong others. Several studies on these oils have called the attention of the pharmaceutical and food pharmaceutical and food industry, since with their use was observed a greater preservation of foods, besides the presence of antimicrobial, analgesic, anti-inflammatory and anti-inflammatory activities, among others[23].

Lavandula hybrida, also popularly known as Lavandin, belongs to the genus *Lavandula* belonging to the Lamiaceae family, descended from the union of the species *Lavandula angustifolia Miller* and *Lavandula latifolia Linn.* The cosmetic and pharmaceutical industry is very interested in this species, since it has an antimicrobial potential against gram positive and gram negative bacterias, as scientifically evidenced, but this effect and mechanism of action[24].

METHODOLOGY

Year and place of study

The laboratory tests were performed in the Microbiology and Biochemistry laboratories of the Federal University of Campina Grande, Patos campus (CSTR), Paraíba state - Brazil, between the years 2021-2022.

MATERIAL AND METHODS

In vitro assays

Test substance

The *Lavandula hybrida Grosso* essential oil was purchased from Indústria Harmonie Aromatherapy® (Florianópolis - SC). In order to perform the pharmacological tests, the substance was solubilized in dimethyl sulfoxide (DMSO) and diluted indistilled water. The concentration of DMSO used was less than 0.1% v/v.

Microorganisms

The bacterial *Staphylococcus saprophyticus* SA45 was used, which was maintained on Muller-Hinton (AMH) Agar at 4°C. The inocula were obtained from overnight cultures on AMH at a temperature ranging from 35°C to 37°C and diluted in sterile sterile saline to obtain a final concentration of approximately 1.5 x 10^8 colony forming units per ml (CFU/ml), adjusted by turbidity compared to the 0.5 tube on the McFarland scale[25].

Culture Media

The culture media used in the assays to evaluate the antimicrobial activity were the Muller Hinton liquid medium and the Muller Hinton agar solid medium. The culture medium was purchased from Difco® and prepared according to the manufacturer's instructions.

Determination of the MIAC (Minimum Inhibitory Adherence Concentration)

The Minimum Adherence Inhibitory Concentration (MIAC) of lavender essential oil lavender essential oil was determined in the presence of 5% sucrose, according to Albuquerque *et al* (2010) [26] with modifications, using concentrations corresponding to the compound from 1:1 up to dilution 1:1024. From the bacterial growth, the strain of *Staphylococcus saprophyticus* strain was grown at 37°C in Mueller Hinton broth (DIFCO, Michigan, USA), then United States), then 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. The incubation was performed at a temperature ranging from 35 to 37°C for 24 hours with tubes tilted at 30º. The reading was

performed by visual observation of the adherence of the bacteria to the walls of the tube after agitation. The assay was performed in duplicate. The same[34] procedure was performed for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA). The MIAC was considered the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube.

RESULTS AND DISCUSSION

Essential oils normally show greater efficacy against gram-positive bacteria than gram-negative ones[27]. This is due to the structures present in gram-positive bacteria, for example, contain a cell wall that allows entry of hydrophobic of hydrophobic molecules that can act both on the cell wall and in the cytoplasm. Gram-negative bacteria, on the other hand, have a more complex cell wall and na outer membrane, which is composed of a double layer of phospholipids, which the inner membrane by means of lipopolysaccharides (LPS), which are responsible for the gram-negative bacteria resistance to antimicrobial compounds[28,29].

Table No. 1: Minimum Inhibitory Adherence Concentration (MIAC) in µg/mL of *Lavandula hybrida Grosso* essential oil and 0.12% Chlorhexidine digluconate against strain of *S. saprophyticus*.

***Lavandula hybrida Grosso* essential oil**										
µg/ml	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:512	1:1024
+++	++	-	-	-	-	-				
0, 12% Chlorhexidine digluconate										
µg/ml	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:512	1:1024
+	++	-	-	-	-	-	-	-		

Regarding the anti-adherent potential, we observe the values in table 3, in which the *Lavandula Hybrida Grosso* essential oil obtained a MIAC value equal to 1:16 compared to chlorhexidine digluconate 0.12%, which had a value of 1:4. That is, to inhibit bacterial adhesion to the tube, it was necessary to use a concentration chlorhexidine, which is the gold standard used in dentistry for chemical control of biofilm.

Biofilm control in the oral cavity is done mechanically, by daily brushing to destabilize the biofilm, daily brushing to destabilize this cluster of microorganisms. This mechanical control can be associated with chemical control that will act mainly as antimicrobial as an antimicrobial agent, such as dentifrices [30,31,32]. In addition, another chemical compound with great effectiveness is chlorhexidine, which has a broad spectrum against bacteria, preventing their growth or formation[33].

However, when used for long periods (over 30 days) chloredixin in excess can cause complications to the body reported for chronic use are dental and mucosal staining, changes in taste, mouth burning, and the appearance of ulcers[33].

Thus, the essential oil of *Lavandula hybrida* has proven to be an alternative with relevant anti-adherent potential. As shown in a study conducted by Gomes *et al* (2021)[34], in which it was possible to observe good nonstick activity of *Lavandula hybrida Grosso* against strains of *Staphylococcus aureus*, where it was necessary a concentration of the essential oil 4 times lower than the concentration of chlorhexidine to prevent biofilm formation in the glass tube.

The results of this current study corroborate a study conducted in 2021 using the same methodology as this study, where it was possible to prove the efficacy of of the anti-adherent activity of *Lavandula hybrida Grosso* against the strain of *Escherichia coli*, which presented a MIAC of 1:16, while chlorhexidine was 1:8. In other words, it took twice as low a concentration of lavender compared to chlorhexidine was required to inhibit microbial adhesion in the tube[35]. Thus, it can be considered a relevant alternative to the alterations presented by chlorhexidine, which is the gold standard in the chemical control of oral biofilm.

In the study conducted by Souza *et al.,* (2018) [36], it aimed to evaluate the anti-adherent potential of *lavandin* essential oil against strains of *Klebsiella pneumoniae* and used 0.12% chlorhexidine digluconate as a positive control. It was possible to observe that the essential oil of *L. hybrida* presented a MIAC of 1:32, while chlorhexidine had a MIAC of 1:8, in other words, chlorhexidine needed a higher concentration for concentration to inhibit biofilm adherence to the glass tube.

CONCLUSION

Lavandula Hybrida Grosso essential oil showed a strong minimal inhibitory activity of adherence *in vitro* against the strain of *Staphylococcus saprophyticus*, which is found in bacterial biofilm, making it a therapeutic option for oral infections caused by this pathogen. However, more research is needed to emphasize its efficacy against different types of microorganisms and then in vivo studies to evaluate its behavior in the body organism.

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