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Analysis of The Anti-Adherent Activity of The Essential Oil from Leaves and Berries of *Juniperus communis* Against Clinical Strains of *Klebsiella pneumoniae*

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ABSTRACT

Klebsiella pneumoniae is one of the bacteria associated with nosocomial infections and in biofilm form, it inhibits the proximity of antimicrobial peptides. The essential oil of *Juniperus communis* is commonly used in traditional medicine, being referred for presenting strong antibacterial, antifungal and anti-inflammatory properties. The work aimed to evaluate the antiadherent activity of the essential oil from leaves and berries of *Juniperus communis* against strains of *Klebsiella pneumoniae*. As for the method, the anti-adherent activity was determined from the Minimum Inhibitory Adherence Concentration (MIAC) of the oil, this was determined in the presence of 5% sucrose, using concentrations corresponding to the pure essential oil until dilution 1:1024. In relation to the results, it was observed that both the essential oil and the 0.12% chlorhexidine digluconate did not present a CIMA value. Concluding that the essential oil of *Juniperus communis* does not present an anti-adherent activity against *Klebsiella pneumoniae* with the methodology used.

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INTRODUCTION

Nosocomial infections occur in any inpatient or outpatient setting and appear 48 hours after admission, within 30 days after receiving health care or up to 90 days after certain surgical procedures (1, 2). Among the most prevalent nosocomial infections, pneumonia, surgical and urinary tract infections as well as gastrointestinal and bloodstream infections stand out (3).

Nosocomial pneumonia is acquired in the lower respiratory tract by aspiration of secretion present in the oropharynx, by inhalation of contaminated aerosols or by hematogenous dissemination originating from a distant focus (4). When associated to mechanical ventilation (MVAP), the breakdown of oral homeostasis favors the formation of a more complex biofilm, colonized by microorganisms resistant to antimicrobial agents and immunological events, mainly respiratory pathogens (5).

Regarding microorganisms more associated to nosocomial pneumonia, gram-negative bacilli stand out, such as *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter* spp. and *Proteus mirabilis*, besides gram-positive cocci, such as *Staphylococcus aureus* (5-9).

Klebsiella pneumoniae, a gram-negative bacterium that belongs to the normal flora of the human mouth and intestine, presents a high rate of hospital infection mainly in hosts whose defenses are impaired. Among these, there are patients with diabetes mellitus, hepatobiliary disease, renal insufficiency and patients under glucocorticoid therapy (10).

Studies report that the increased mortality rate from infections caused by *Klebsiella pneumoniae* is mainly due to sepsis, septic shock, bloodstream infections and inadequate antimicrobial therapy (11). The exacerbated use of antibiotics in the hospital environment generates a high bacterial proliferation and resistance. This resistance is related to the production of enzymes, which are classified according to their action against antibiotics (12).

Among these enzymes, the carbapenase stands out, an enzyme produced by *Klebsiella pneumoniae*, which plays an important role in resistance to carbapenem antibiotics, making therapeutic options very limited (13).

Knowledge about bacterial resistance, its mechanisms of occurrence, prevention and control strategies is of great importance, given that *K. pneumoniae* in the biofilm form protects itself from the host immune response, as well as inhibiting the proximity of antibodies and peptide antimicrobials (14, 15).

However, the increase in the number of researches with natural products in dentistry, based on the search for biologically active compounds that cause less toxicity and less drug interactions, have contributed to phytotherapy gaining space as a treatment option for several oral diseases and to control the formation of dental biofilm, which when controlled, contributes significantly in the treatment of systemic diseases of patients in ICU (16-18).

Essential oils (EOs) are complex mixtures of low molecular weight compounds extracted from plants by steam distillation using various solvents. Their main constituents are terpenes, which can be classified as sesquiterpenes, monoterpenes and diterpenes based on the number of isoprene units present in their chemical structure. These components provide characteristic aroma and biological properties to EOs, such as antibacterial, antifungal, antiseptic, anti-inflammatory, anticancer, antimutagenic, antidiabetic, antiviral and antiprotozoal activities (19, 20).

Juniperus communis essential oil is an aromatic and volatile liquid accumulated in cones (berries) and leaves (needles). It is commonly used in traditional medicine and by the pharmaceutical industry as it is referred to for its anti-inflammatory, analgesic, anti-rheumatic, diuretic, carminative, digestive and antioxidant activity (21 -23).

However, the presence of monoterpene hydrocarbons in *Juniperus communis* essential oil, with α-pinene being the majority compound, followed by β-myrcene and sabinene in the composition of the essential oil of the berries compared to that derived from juniper needles, with predominance in limonene, β-felandrene, α-pinene and sabinene, justifies the study of its antimicrobial activity (24, 25). In studies already carried out, it has been verified that it presents strong bactericidal activity against gram-positive bacteria, being this activity superior to the activity exerted against gram-negative bacteria (26).

In view of the information regarding the therapeutic potential of essential oils and the importance

in the combat of infections caused by multiresistant bacteria, this work intends to evaluate the anti-adherent activity of the essential oil of the leaves and berries of *Juniperus communis* against strains of *Klebsiella pneumoniae*.

METHODOLOGY

Year and place of the 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 e Métodos.

In vitro tests

Test substance

The essential oil from Juniperus communnis leaves and berries was purchased from Indústria Quinari ® (Ponta Grossa - PR). To perform the pharmacological tests, the substance was solubilized in DMSO (dimethylsulfoxide) and tween 80, as well as diluted in distilled water. The concentration of DMSO used was less than 0.1% v/v. The project followed the rules of CGEN - Genetic Heritage Management Council, registered on the SISGEN platform under protocol number A2E529D.

Micro-organisms

A clinical strain of *Klebsiella pneumoniae* (Kp105) was used and maintained in Muller-Hinton Agar (MH) at 4ºC. The inoculum was obtained from overnight culture in MH at 35ºC - 37ºC and diluted in sterile saline solution to obtain a final concentration of approximately 1.5 x 108 colony forming units per ml (CFU/ml), adjusted by turbidity comparing with the 0.5 tube of the McFarland scale (27).

Culture Media

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

Determination of the CIMA (Minimum Inhibitory Adherence Concentration)

The Minimum Inhibitory Adherence Concentration (MIC) of *Juniperus communis* essential oil was determined in the presence of 5% sucrose, according to Albuquerque and colleagues. (28) with modifications, using concentrations corresponding to the compound up to 1:1024 dilution. From the bacterial growth, the clinical strain of *Klebsiella pneumoniae* (Kp105) was cultivated at 35ºC - 37 ºC in Mueller Hinton broth (DIFCO, Michigan, United States), then 0.9 ml of the subculture was distributed in test tubes and then 0.1 ml of the solution corresponding to the essential oil dilutions was added. Incubation was carried out at 35ºC - 37 ºC for 24 hours with tubes inclined at 30º. The reading was performed by visual observation of the adherence of the bacteria to the walls of the tube after shaking it. The test 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.

RESULTS AND DISCUSSION

The results found about the anti-adherent activity are described in table 3, where both the essential oil of the leaves and berries of *Juniperus communis*, and the 0.12% chlorhexidine digluconate did not show a MIAC value.

Table No. 1. Minimum Inhibitory Adherence Concentration (MIAC) in µg/mL of *Juniperus communis* leaf and berry essential oil and 0.12% Chlorhexidine digluconate against clinical strain of *K. pneumoniae*.										
***Juniperus communis* essential oil**										
µg/ml	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:512	1:1024
	-	-	-	-	-	-	-	-	-	-
Chlorhexidine digluconate 0.12%										
µg/ml	1:1	1:2	1:4	1:8	1:16	1:32	1:64	1:128	1:512	1:1024
	-	-	-	-	-	-	-	-	-	-

Legend: (+) No adhesion to tube wall (-) With adhesion to tube wall

Essential oils promote disruption of the bacteria cell wall by disrupting the arrangement of different fatty acids, phospholipid bilayers and polysaccharide molecules. In addition, cellular proteins can also be affected, as well as the inactivation of essential enzymes (29, 30). Thus, these compounds have been widely investigated for their anti-adherent property, being therefore an alternative to the use of chlorhexidine because of its adverse effects, such as taste alteration, dental staining and gum scaling (31). In a study carried out by Souza and colleagues. (32), which analyzed the antiadherent activity of the essential oil of Lavandula híbrida grosso against strains of *Klebsiella pneumoniae*, it was observed a high antiadherent potential of this essential oil, since it was 4 times more potent than the chlorhexidine digluconate 0.12%. Ramalho and colleagues. (33), analyzed the anti-adherent activity of the essential oils of *Eucalyptus globulus* and *Eucalyptus citriodora* against strains of *Klebsiella pneumoniae*. According to the results, only the essential oil of *Eucalyptus globulus* showed similar activity to chlorhexidine digluconate 0.12%, and may be an alternative method in the control and formation of biofilms of *Klebsiella pneumoniae*. However, the essential oil of *Eucalyptus citriodora* did not show anti-adherent activity against the bacteria. Another study by Peruč and colleagues. (34) also obtained positive results regarding the anti-adherent activity of the essential oil of *Juniperus communis*. Such compound inhibited the formation of biofilms of strains of *Mycobacterium avium* and *Mycobacterium intracellulare* because the high content of lipids in the extracellular matrix of the

biofilm of the mycobacteria facilitated the diffusion of the essential oil. The present work suggests the use of new methods to investigate the anti-adherent activity of the essential oil of the leaves and berries of *Juniperus communis*, since it was ineffective against the *Klebsiella pneumoniae* strain.

CONCLUSION

As for the anti-adherent effect of the essential oil from the leaves and berries of Juniperus communis, new tests are necessary, for although it has not shown any effect with the methodology used, its therapeutic potential against other microorganisms or even its association with conventional drugs in order to potentiate its effect should not be disregarded.

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