Evaluation of the Antibacterial Activity of 
*Gaultheria procumbens* Essential Oil Against 
*Klebsiella pneumoniae* Strains

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**ABSTRACT**

The oral biofilm contains a wide variety of microorganisms, mostly bacteria, which are responsible for the development of various infections, such as periodontal disease. Periodontal disease is of infectious and inflammatory origin, caused by the present in the biofilm. It acts by destroying the protective and supporting tissues of the dental elements, involving local, systemic, environmental, and genetic factors. Studies show that periodontal disease is directly related to various systemic morbidities, among which we can highlight respiratory problems, where nosocomial pneumonia has stood out, as there is a relationship between it and the microorganisms present in the oral cavity, among which we can highlight *Klebsiella pneumoniae*. The resistance of this bacteria to antimicrobials has become increasingly frequent, so the search for alternative treatments has been increasing, among them natural products, especially essential oils, as they have numerous therapeutic activities against various microorganisms, among them we will highlight the essential oil of *Gaultheria procumbens* which has antimicrobial, anti-adherent, anti-inflammatory activities, among others. The aim of this research is to analyze the possible antimicrobial and anti-adherent potential of *Gaultheria procumbens* essential oil against *Klebsiella pneumoniae* strains. To carry out this research, the Minimum Inhibitory Concentration (MIC) technique was used, which was determined by the microdilution technique in plates containing 96 sterile holes the Minimum Bactericidal Concentration (MBC) will be read 48 hours after the MIC, using plates with 96 holes. The Minimum Adherence Inhibitory Concentration (MIC) was carried out in glass tubes, using a medium with 5% sucrose. This research showed that the Minimum Inhibitory Concentration (MIC) ranged from 256 μg/mL to 512 μg/mL, and the Minimum Bactericidal Concentration (MBC) ranged from 1024 μg/mL to 256 μg/mL. It showed strong and moderate antibacterial, bactericidal and bacteriostatic effects and no anti-adherent effect. The strains tested showed strong and moderate effects on the MIC, and bactericidal and bacteriostatic effects on the MBC, and no anti-adherent effect on the MIC.

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1. **Introduction**

There are several species of bacteria in the oral cavity, some of which are responsible for forming the biofilm that adheres to tooth surfaces [1]-[6]. As a result, there are several factors that lead the biofilm to enter into dysbiosis, which causes it to become unbalanced, leading to manifestations of oral infections, and it is necessary to control the biofilm so that it enters into symbiosis and avoid the proliferation of pathogenic bacteria [7].

Various oral pathologies can be caused by the accumulation of biofilm, the most frequent of which are caries, periodontal diseases, and halitosis. Regular removal of supragingival and subgingival biofilm can be considered the main factor in the prevention and treatment of these diseases; in addition, removal of supragingival biofilm has been shown to be effective in preventing gingival inflammation and the development of periodontitis. Thus, brushing and disorganizing the biofilm is of the utmost importance to prevent the oral cavity from becoming unbalanced, so it is necessary to emphasize that hygiene is extremely important to avoid any pathology caused by the accumulation of biofilm [8].

Biofilm can undergo changes in its composition, especially in hospitalized individuals, which can lead to the production of pathogens, including respiratory pathogens, among which we can highlight *Klebsiella pneumoniae*, which causes an infectious process inherent to nosocomial pneumonia [1]-[6].

*Klebsiella pneumoniae*, a gram-negative bacterium, is considered pathogenic and can cause urinary tract infections, respiratory infections, and bacteremia. It forms biofilms on medical devices such as catheters, which is why it causes many nosocomial infections [9].

The overuse of antibiotics and self-medication has grown a lot in recent years, and this has created a problem for the population, as pathogens create antibiotic resistance. There are multi-resistant bacteria that put human health at risk, and the effectiveness of antibiotics against them is already questionable as a result of their overuse [10].

It is also worth highlighting the use of medicinal plants, which were used in the past by the natives to treat infectious diseases. It is well known that the search for treatments using natural products has been increasing over time, given that medicinal plants have numerous pharmacological activities, as well as their low cost and acceptance by the population [11], [12].

There are also essential oils, which are natural products obtained from the secondary metabolism of plants. Their characteristic is their pleasant smell, which attracts pollinating animals, and they help to separate seeds, as well as help to defend plants. Oils are liquid, volatile, and lipophilic and can be found in various parts of the plant, such as flowers, leaves, roots, and fruit. The study of oils has aroused the interest of the pharmaceutical and food industries, as their use helps to preserve food better, and the pharmaceutical industry has noted that they have antimicrobial, analgesic, anti-inflammatory, and antiviral activities, among others [13].

*Gaultheria procumbens* is an *Ericaceae* species native to North America. It is a small shrub with green leaves and red fruits that have aromatic characteristics. It is a medicinal plant that is widely used to treat inflammation, rheumatoid arthritis, swelling pains, chronic tracheitis, and acute and chronic constipation. Its analgesic and anti-inflammatory activities are derived from salicylic acid, especially methyl salicylate (essential oil), which has antioxidant effects. However, few studies have reported the antibacterial activity of the essential oil of this species [14].

2. **Objectives**

2.1. **General**

To evaluate the antibacterial and anti-adherent activity of *Gaultheria procumbens* essential oil against strains of *Klebsiella pneumoniae*.

2.2. **Specific**

- Determine the Minimum Inhibitory Concentration of the essential oil against the different strains of *Klebsiella pneumoniae*;
- Verify the Minimum Bactericidal Concentration of *Gaultheria procumbens* essential oil against strains of *Klebsiella pneumoniae*.

3. **Methodology**

3.1. **Place of Study**

The laboratory tests were carried out in the Microbiology and Biochemistry laboratories of the Federal University of Campina Grande, Patos campus (CSTR), Paraíba state-Brazil.

3.2. **Test Substances**

*Gaultheria procumbens* essential oil was purchased from Indústria Harmonie Aromaterapia® (Florianópolis-SC). For the pharmacological tests, the substance was solubilized in DMSO and diluted in distilled water. The concentration of DMSO (dimethyl sulfoxide) used will be less than 0.1% v/v. The project follows the rules of CGEN - the Genetic Heritage Management Council, and is registered on the SISGEN platform under protocol number AAD241D.

3.3. **Bacterial Species and Culture Medium**

The following strains of *Klebsiella pneumoniae* (ATCC 13883, Kp 101, Kp 102, Kp 103, Kp 104, and Kp 105) will be used. The inocula were obtained from overnight cultures in MH at 37 °C and diluted in sterile saline solution to obtain a final concentration of approximately 1.5 × 108 colony-forming units per ml (CFU/ml), adjusted for turbidity by comparing with the 0.5 tube of the McFarland scale.
The culture media used in the tests to assess antimicrobial activity were Muller Hinton liquid medium (MH) and Muller Hinton Agar solid medium (AMH). The culture medium was purchased from Difco® and prepared according to the manufacturer’s instructions.

3.4. Determination of the Minimum Inhibitory Concentration (MIC)

The MIC was determined using the microdilution technique in a 96-well plate with a U-shaped bottom. In a 96-well plate, 100 μl of double-concentrated Mueller Hinton broth and 100 μl of Gaultheria procumbens essential oil were added at concentrations of 1024 to 4 μg/ml. The MIC was determined with 10 μl of the microorganism in each cavity, approximately 1.5 × 108 UFC/ml. The penultimate well containing 200 μl of broth was inoculated with the microorganism suspension, being the growth control, and the last well-received only 200 μl of broth, being the negative control. The test was carried out in duplicate. The plates were incubated at 35 °C for 24 hours. After the appropriate incubation time for the tests with the bacteria, the first reading of the results was taken. Next, 20 μl of resazurin sodium solution (SIGMA) was added to sterile distilled water at a concentration of 0.01% (w/v), recognized as a colorimetric oxide-reduction indicator for bacteria. The reading was made visually by the absence or presence of growth of the microorganism through the formation of a cluster of cells (bud). The color of the solution also changed from blue to pink, indicating growth. A further incubation was carried out at 35 °C + or −2. The MIC was determined as the lowest concentration of essential oil that inhibited the visible growth of the microorganism and also by observing the change in color of the solution from blue to pink, indicating the growth of the microorganism [15]–[18].

3.5. Determination of the Minimum Bactericidal Concentration (MBC)

After reading the results, inoculations (10 μl) of three dilutions from the MIC were made into Mueller-Hinton broth medium (100 μl/cavity) in a sterile microdilution plate to determine the MBC. After incubation at 35 °C + or −2 for 24 hours, 20 μl of resazurin was added. The tests were incubated at 37 °C for a further 24 hours to confirm the concentration capable of inhibiting the total growth of the bacterial species, which was verified by a non-change in the color of the indicator dye [19]–[20].

4. RESULTS AND DISCUSSION

The antimicrobial activity of essential oils is assessed through the lowest concentration of the test substance required to inhibit the growth of the exposed microorganism; this value is known as the Minimum Inhibitory Concentration (MIC). In this experiment, the essential oil of Gaultheria procumbens (Whintergreen) had an antibacterial effect on five of the six strains of Klebsiella pneumoniae with MIC values ranging from 256 μg/mL to 512 μg/mL, and the sixth strain had a MIC greater than 1024, as shown in Table I.

The minimum bactericidal concentration (MBC) aims to assess whether the substance being analyzed is capable of killing or inhibiting the growth of bacteria. The results of the minimum bactericidal concentration (MBC) ranged from 1024 to 256 μg/mL. As shown in the Table II.

As proposed by Sartoratto et al. [21] (2004), the antimicrobial activity of essential oils is classified as strong when they have a MIC of up to 500 μg/mL, moderate for MIC values between 600 and 1500 μg/mL, and weak for MICs above 1500. Thus, the results identified in this study show that the essential oil of Gaultheria procumbens (Whintergreen) has a strong inhibition against the Klebsiella pneumoniae ATCC strain and Klebsiella pneumoniae 101, while Klebsiella pneumoniae 102, 103 and 104 showed moderate inhibition and Klebsiella pneumoniae 105 showed no inhibition.

According to Hafidh et al. [22], for a substance to be considered bactericidal or bacteriostatic according to its MIC, it must be equal to or twice as high as the MIC or the MIC must be greater than twice the MIC. Therefore, the results found in this study show that the essential oil of Gaultheria procumbens (Whintergreen) has bactericidal potential against the ATCC strain and bacteriostatic potential against strains 101, 102, 103 and 104.

Corroborating the data in this research, the study by Nicolik et al. [23] showed the antimicrobial effect of Gaultheria procumbens essential oil against a wide range of Gram-positive and Gram-negative bacteria and fungi, as well as good antioxidant and anti-radical potential. In addition, the essential oil inhibited the growth of all the microorganisms tested, including Streptococcus mutans orally, inhibiting the formation of biofilm, and was also able to inhibit fungi such as Candida albicans. In this study, eight components were found with methyl salicylate being the most dominant in the essential oil of G. procumbens, which may lead to its antibacterial effect.

The study by Verdi et al. [24] also showed the antibacterial activity of the essential oil of G. procumbens. To carry out the present study, the same MIC and MBC techniques used in this study were used, and the results showed efficacy against various microorganisms, including Aeromonas caviae, Candida albicans, and Mycobacterium fortuitum.

As well as the study cited that the essential oil of Gaultheria procumbens has an anti-inflammatory effect, Michel et al. [14] research shows that the dried leaf stratum of G. procumbens contains considerable amounts of antioxidant polyphenols, which have significant dose-dependent SET and HAT antioxidant capacities in vitro, and also has moderate anti-inflammatory activity, which is related to its phenolic composition. In view of this, phenolic acids are mainly responsible for the antioxidant capacity tested in the study, while flavonoids are the main determinants of the anti-inflammatory effects.

In the study by Olszewska et al. [25], also that the plant species Gaultheria procumbens can be a plant used in phytotherapy, its aerial parts, leaves, and stems are rich in polyphenols with anti-inflammatory and antioxidant effects, so in the study the extracts of these parts were used. With this, it was able to inhibit two important pro-inflammatory cyclooxygenase enzymes (COX-2
and hyaluronidase), and most of them, except gaultherin, exerted potent direct antioxidant activity. In addition, it showed cell safety, which was confirmed for all the compounds by flow cytometry.

5. Conclusion

This research shows that the essential oil of Gaultheria procumbens (Whitewatergreen) had an antibacterial effect, with bactericidal and bacteriostatic characteristics, against the strains tested in the methodology applied. However, more studies need to be carried out to confirm this potential, especially in vivo studies.

Conflict of Interest

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

References


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