

Evaluation of the Anti-Bacterial and Anti-Adherent Activity of *Pogostemon Cablin's* Essential Oil Against *Klebsiella Pneumoniae*

Vinícius Rocha Lima Santos^{1,*}, Abrahão Alves de Oliveira Filho²,
Raline Mendonça dos Anjos², Elizandra Silva da Penha², Gymenna Maria Tenório Guenes²,
Camila Helena Machado da Costa Figueiredo², Emanuel Vitor Alves da Silva¹,
Elaine Bezerra de Oliveira¹, Roberta Gomes Pereira da Silva¹,
Lara Mayanne Moreira de Oliveira Nóbrega¹, Ozanna Soares Medeiros de Araújo¹,
and Iano Goveia de Araújo¹

ABSTRACT

Klebsiella pneumoniae is an example of a microorganism that does not belong to the oral biofilm in a state of health but is present in cases of infection, especially nosocomial infections. The high virulence and resistance of this bacterium to various forms of antimicrobial treatment represent a major concern in the hospital environment, as it is responsible for a high number of deaths of hospitalized patients under mechanical ventilation, so the search for new drugs that are effective in treating this bacterium is extremely important. Natural products, especially essential oils, have considerable therapeutic activities in antimicrobial treatments. A good example is *Pogostemon cablin*, which has antimicrobial, anti-adherent, and anti-inflammatory activities, among others. Thus, this research aimed to analyze the possible antimicrobial potential of the essential oil extracted from *Pogostemon cablin* against strains of *Klebsiella pneumoniae*, using the Minimum Inhibitory Concentration (MIC), which was determined using the microdilution technique in plates containing 96 sterile holes with positive control, the antimicrobial chloramphenicol. The Minimum Bactericidal Concentration (MBC) was read 48 hours after the MIC, using plates with 96 holes. After the reading, it was found that the essential oil of *Pogostemon cablin* had a 50% MIC of 512 µg/mL against the *Klebsiella pneumoniae* strains tested, making it a strong bacteriostatic. It was therefore possible to observe the antibacterial potential of *Pogostemon cablin* essential oil against the *K. pneumoniae* strains tested.

Submitted: August 30, 2023

Published: December 20, 2023

doi: 10.24018/lejmed.2023.5.6.1928

¹Dentistry Student at the Federal University of Campina Grande–UFCG, Brazil.

²Master Professor at the Federal University of Campina Grande–UFCG, Brazil.

*Corresponding Author:
e-mail: rocha.lima@estudante.ufcg.edu.br

Keywords: Microbiology, Odontology, Phytotherapy.

1. INTRODUCTION

The microbiota present in the oral cavity is the second most divers in the human body, containing more than 700 bacterial species, most of which maintain a symbiotic relationship with the host's organism [1]. The human oral cavity is a very complex environment that contains various substances and microorganisms, their organization into communities surrounded by a polysaccharide matrix containing nucleic acids, proteins, and water forms the biofilm, which in turn is adhered to the teeth, dental restorative

structures, and soft tissues of the oral cavity. Each microorganism present in the biofilm has individual characteristics and functions in this structure, so much so that the biofilm is often compared to multicellular organisms, where the constituent cells differentiate with specialized functions and work together for the survival of the community [2], [3].

Each cubic millimeter of oral biofilm contains approximately 100 million microorganisms, including bacteria, fungi, and viruses, so biofilm can act as a reservoir for pathogens that can later enter the bloodstream and/or



remain present in saliva and be aspirated, causing infections in other body parts [4].

Klebsiella pneumoniae is a bacterium present in the gastrointestinal biofilm, but several studies point to its presence in the oral biofilm in specific situations, such as hospitalized, immunocompromised patients, in ICUs, and under mechanical ventilation [5], [6]. Bacteria belonging to the genus *Klebsiella* are the main causes of hospital infections with high morbidity and mortality, and *Klebsiella pneumoniae* is the species of greatest clinical importance since it can cause infections in the urinary system, septicemia and nosocomial pneumonia [7]. Nosocomial pneumonia is the second most common nosocomial infection, and the one that causes the most deaths among nosocomial infections, where up to half of those affected can die [8]. A very important point about *Klebsiella pneumoniae* is its high resistance to various antibiotics, representing one of the most worrying pathogens when it comes to bacterial resistance, which causes an alert not only in the treatment of infections caused by this bacterium but also in the development of new drugs, especially substances from medicinal plants that are effective in antibacterial action against *Klebsiella pneumoniae* [6]–[9].

Essential oils are natural products obtained from the secondary metabolism of medicinal plants, acting in plant reproduction by attracting pollinating animals, as well as in plant defense. They are volatile, lipophilic liquids that can be found in flowers, leaves, roots, fruits, and other parts of the plant. Several studies on these oils have attracted the attention of the pharmaceutical industry, as they have been found to have antimicrobial, analgesic, and anti-inflammatory activities, among others [10].

A viable alternative would be the essential oil from *Pogostemon cablin* (Blanco), better known as patchouli, which is an aromatic herb native to Southeast Asia and is widely cultivated in countries such as Indonesia, the Philippines, Malaysia, China, and Brazil. In China and the surrounding regions, *P. cablin* is popular for treating colds, headaches, fever, vomiting, indigestion, and diarrhea, and is also widely used as an antifungal agent [11]–[13].

The essential oil extracted from this plant is rich in terpenes, with patchoulol as its main constituent. Several studies show that this oil has antimicrobial, antioxidant, analgesic, anti-inflammatory, antimutagenic, antithrombotic, antiemetic, and cytotoxic properties, and also that the ethanolic extract of *P. cablin* has moderate inhibitory and antimicrobial action against various species responsible for nosocomial infections, such as *Staphylococcus aureus*, MRSA (methicillin-resistant *Staphylococcus aureus*) and *Streptococcus pyogenes*.

The main objective of the article is to evaluate the antibacterial activity of the essential oil of *Pogostemon cablin* against *Klebsiella pneumoniae* strains.

2. METHOD AND VIABILITY

2.1. Test-Substance

The essential oil from *Pogostemon cablin* 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 (dimethylsulfoxide) used was less than 0.1% v/v. The project followed the rules of CGEN- Conselho de Gestão do Patrimônio Genético (Genetic Heritage Management Council) and was registered on the SISGEN platform under protocol number A4F2097.

2.2. Bacterial Species and Culture Medium

The following strains of *Klebsiella pneumoniae* (ATCC 13883, Kp 101, Kp 102, Kp 103, Kp 104, Kp 105) were used. The strains were kept in Muller Hinton Agar (MHA) at 4 °C and 24-hour replicates in MHA incubated at 35 °C were used for the tests. For the antimicrobial activity study, a bacterial inoculum of approximately 1.5×10^8 CFU/mL was used, standardized according to the turbidity of the 0.5 tubes of the McFarland scale [14], [15].

2.3. 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 thyme essential oil were added at concentrations of 1024 to 16 µg/ml. The MIC was determined with 10 µl of the microorganism in each cavity, approximately 1.5×10^8 CFU/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. The solution was incubated again at 37 °C. 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 growth of the microorganism [16]–[19].

2.4. Determination of the Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of the oil was also determined for the bacterial strains. After reading the MIC in 24 hours, aliquots of 20 µL were taken from each well of the microtiter plate that showed no bacterial growth and transferred to wells of a new microtiter plate containing 100 µL of Muller Hinton broth, devoid of any antimicrobial. The inoculated plates were aseptically closed and incubated at 35 °C, and the CBMs were recorded after 48 h. The CBM was defined as the lowest concentration of the essential oil that resulted in visible inhibition of the growth of the microorganism [20], [21].

TABLE I: MINIMUM INHIBITORY CONCENTRATION (MIC) RESULTS

	KP ATCC	KP 101	KP 102	KP 103	KP 104	KP 105
1024 µg/mL	+	+	+	+	+	*
512 µg/mL	-	+	+	+	+	*
256 µg/mL	-	-	-	-	+	*
128 µg/mL	-	-	-	-	-	*
⋮						
⋮						
[22] 4 µg/mL	-	-	-	-	-	*
Negative control	-	-	-	-	-	-
Positive control	+	+	+	+	+	+

Note: (+) Inhibition (-) No inhibition (*) above 1042 µg/mL.

TABLE II: MINIMUM BACTERICIDAL CONCENTRATION (MBC) RESULTS

	KP ATCC	KP 101	KP 102	KP 103	KP 104
1024 µg/ml	-	-	-	-	-
512 µg/mL	-	-	-	-	-
256 µg/mL	-	-	-	-	-
128 µg/mL	-	-	-	-	-

Note: (+) Inhibition (-) No inhibition.

2.5. Viability

All the equipment used to carry out this research can be found in the Microbiology laboratory at UFCG's Center for Health and Rural Technology, which makes it feasible. In addition, all the reagents will be purchased with the researcher's own funds.

3. RESULTS AND DISCUSSION

3.1. Minimum Inhibitory Concentration (MIC) Results

The evaluation of the essential oil's MIC showed a 50% MIC value of 512 µg/mL according to Table I.

Taking into account the scale of Sartoratto *et al.* [23] where the inhibitory action of oils is classified as strong with values up to 500 µg/ml, moderate between 600 and 1500 µg/ml and weak above 1500 µg/ml, based on this statement it is observed that the oil had a strong antibacterial effect. Other studies on the same oil corroborate its antibacterial effect, for example the study by Das *et al.* [24] which demonstrated a strong inhibitory effect against strains of *Bacillus subtilis*, *Bacillus cereus*, *Salmonella Paratyphi*, *Pseudomonas aeruginosa*, *Shigella dysenteriae* and *Escherichia coli*. The same study also tested the inhibitory effect of the oil against fungal species, where the oil showed strong inhibitory action against *Aspergillus fumigatus*, a fungal species that causes allergic bronchopulmonary aspergillosis (ABPA) [25].

There are currently few *in vivo* studies using this oil as a test substance, but in the study by Wan *et al.* [26], which tested the *in vitro* and *in vivo* antimicrobial potential of patchouli alcohol, a compound isolated from the essential oil of *Pogostemon cablin*, it showed great protective action against MRSA, with a survival rate of 100% of the guinea pigs at concentrations of 100 and 200 mg/kg.

3.2. Minimum Bactericidal Concentration (MBC) Results

With regard to CBM, the oil showed values above the concentration of 1024 µg/ml, as shown in Table II.

For a compound to be considered bactericidal, it needs to obtain a CBM value that is double or equal to its MIC value [27], so the compound had a bacteriostatic effect. These data are not in line with the study by Das *et al.* [24] where the oil showed bactericidal action against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Shigella sonnei*, *Shigella dysenteriae*, *Vibrio cholerae* and *Escherichia coli*.

4. CONCLUSION

Therefore, from the data obtained, it was observed that within the methodology used, the essential oil of *Pogostemon cablin* has a strong bacteriostatic effect against the strains of *Klebsiella pneumoniae* tested, but it did not have a bactericidal effect on them. Further studies are needed to evaluate this potential, such as *in vivo* studies.

CONFLICT OF INTEREST

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

REFERENCES

- [1] Kilian M, Chapple IL, Hannig M, Marsh PD, Meuric V, Pedersen AM, *et al.* The oral microbiome—An update for oral health-care professionals. *Br Dent J.* 2016 Nov 18;221(10):657–66. doi: 10.1038/sj.bdj.2016.865. PMID: 27857087.
- [2] Souza JCM, Mota RRC, Sordi MB, Passoni BB, Benfatti CAM, Magini RS. Biofilm formation on different materials used in oral rehabilitation. *Braz Dent J.* 2016 Mar;27(2):141–7. doi: 10.1590/0103-6440201600625.
- [3] André Luis Souza dos S. Brazilian scientists: much to learn from the microbial biofilm lifestyle (a resistant, resilient, well-orchestrated, and dynamic organization). *Ciência & Saúde Coletiva.* 2022;27(3):947–50.

- [4] Damascena LCL, Rodrigues LV, Costa RC, Da Nóbrega JBM, Dantas EL de A, Valença AMG. Factors associated with oral biofilm in ICU patients with infectious diseases. *Rev odontol UNESP*. 2017 Nov;46(6):343–50. doi: 10.1590/1807-2577.0511.
- [5] Souza LCD, Mota VBR, de Carvalho AVDSZ, Corrêa RDGCF, Libério SA, Lopes FF. Association between pathogens from tracheal aspirate and oral biofilm of patients on mechanical ventilation. *Braz oral res*. 2017;31:e38. doi: 10.1590/1807-3107BOR-2017.vol31.0038.
- [6] Vivas R, Dolabella SS, Barbosa AAT, Jain S. Prevalence of Klebsiella pneumoniae carbapenemase-and New Delhi metallo-beta-lactamase-positive K. pneumoniae in Sergipe, Brazil, and combination therapy as a potential treatment option. *Rev Soc Bras Med Trop*. 2020;53:e20200064. doi: 10.1590/0037-8682-0064-2020.
- [7] Pereira SCL, Vanetti MCD. Potential virulence of Klebsiella sp. isolates from enteral diets. *Braz J Med Biol Res*. 2015 Sep;48(9):782–9. doi: 10.1590/1414-431X20154316.
- [8] de Oliveira LCBS, Carneiro PPM, Fischer RG, Tinoco EMB. A presença de patógenos respiratórios no biofilme bucal de pacientes com pneumonia nosocomial. *Rev Bras Ter Intensiva*. 2007 Oct;19(4):428–33. doi: 10.1590/S0103-507X2007000400004.
- [9] Navon-Venezia S, Kondratyeva K, Carattoli A. Klebsiella pneumoniae: a major worldwide source and shuttle for antibiotic resistance. *FEMS Microbiol Rev*. 2017;41(3):252–75.
- [10] Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils—A review. *Food Chem Toxicol*. 2008;46:446–75.
- [11] Chen Y, Wu YG, Xu Y, Zhang JF, Song XQ, Zhu GP, et al. Dynamic accumulation of sesquiterpenes in essential oil of Pogostemon cablin. *Rev Bras Farmacogn*. 2014 Oct;24(6):626–34. doi: 10.1016/j.bjp.2014.11.001.
- [12] Ribeiro AS, Bertolucci SKV, de Carvalho AA, Tostes WN, Coelho AD, Pinto JEBP. Light intensities alter growth and essential oil of patchouli under shade nets. *Cienc Rural*. 2022;52(5):e20210118. doi: 10.1590/0103-8478cr20210118.
- [13] Xu Y, Wu Y-G, Chen Y, Zhang J-F, Song X-Q, Zhu G-P, et al. Autotoxicity in Pogostemon cablin and their allelochemicals. *Rev Bras Farmacogn*. 2015 Mar;25(2):117–23. doi: 10.1016/j.bjp.2015.02.00.
- [14] Cleeland R, Squires E. Evaluation of new antimicrobials in vitro and in experimental animal infections. In: *Antibiotics in Laboratory Medicine*. New York: Willians & Wilkins; 1991. pp. 739–88
- [15] Hadacek F, Greger H. Testing of antifungal natural products: methodologies, comparability of results and assay choice. *Phytochem Anal: An Int J Plant Chem Biochem Techniques*. 2000;11(3):137–47.
- [16] Bona EAMD, da Pinto FGS, Fruet TK, Jorge TCM, de Moura AC. Comparação de métodos para avaliação da atividade antimicrobiana e determinação da concentração inibitória mínima (cim) de extratos vegetais aquosos e etanólicos. *Arq Inst Biol*. 2014 Jul;81(3):218–25. doi: 10.1590/1808-1657001192012.
- [17] Clsi. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. CLSI document M07-A9*. Wayne, PA: Clinical and Laboratory Standards Institute; 2012.
- [18] Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portales F. Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance Mycobacterium tuberculosis. *Antimicrob Agents Chemother*. 2002;46:2720–2722.
- [19] Ostrosky EA, Mizumoto MK, Lima MEL, Kaneko TM, Nishikawa SO, Freitas BR. Métodos para avaliação da atividade antimicrobiana e determinação da Concentração Mínima Inibitória (CMI) de plantas medicinais. *Rev Bras Farmacogn*. 2008 Apr;18(2):301–7. doi: 10.1590/S0102-695X2008000200026.
- [20] Guerra FQS, Mendes JM, Oliveira WA, Costa JGM, Coutinho HDM, Lima EO. Chemical composition and antimicrobial activity of Cinnamomum zeylanicum Blume essential oil on multi-drug resistant Acinetobacter spp. strains. *J Biol Pharm*. 2012;8(1):62–70.
- [21] Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *Afr J Biotechnol*. 2008;7(12):1797–1806.
- [22] Dechayont B, Ruamdee P, Poonnaimuang S, Mokmued0 K, Chunthong-Orn J. Antioxidant and antimicrobial activities of Pogostemon cablin (Blanco) Benth. *J Bot*. 2017;2017:1–6. doi: 10.1155/2017/8310275.
- [23] Sartoratto A, Machado ALM, Delarmelina C, Figueira GM, Duarte MCT, Rehder VLG. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Braz J Microbiol*. 2004 Oct;35(4):275–80. doi: 10.1590/S1517-83822004000300001.
- [24] Das P, Dutta S, Begum J, Anwar MD. Antibacterial and antifungal activity analysis of essential oil of Pogostemon cablin (Blanco) Benth. *Bangladesh J Microbiol*. 2016;30:7. doi: 10.3329/bjm.v30i1-2.28446.
- [25] Latgé JP, Chamilos G. Aspergillus fumigatus and Aspergillo-sis in 2019. *Clin Microbiol Rev*. 2019;33(1):e00140–18. doi: 10.1128/CMR.00140-18.
- [26] Wan F, Peng F, Xiong L, Chen JP, Peng C, Dai M. In vitro and in vivo antibacterial activity of patchouli alcohol from Pogostemon cablin. *Chin J Integr Med*. 2021;27(2):125–30. doi: 10.1007/s11655-016-2452-y.
- [27] Hafidh RR, Abdulmir AS, Vern LS, Bakar FA, Abas F, Jahanshiri F, et al. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. *Open Microbiol J*. 2011;5:96–106. doi: 10.2174/1874285801105010096.