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Evaluation of The Anti-Adherent Activity of Pogostemon Cablin Essential Oil Against the Clinical Strain of *Klebsiella oxytoca*

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ABSTRACT

Klebsiella oxytoca (*K. oxytoca*) has been isolated from different clinical samples. It is a bacterium capable of forming biofilms in most environments, as an opportunistic pathogen that colonizes hospital equipment and has shown great resistance to conventional treatment methods. Thus, products of natural origin have gained prominence in the scientific field. An example of this is the essential oil of *Pogostemon cablin* (*P. cablin*), which due to its great phytochemical potential has been the target of much research, highlighting its antimicrobial activity. Given the above, this work aims to evaluate the anti-adherent activity of the essential oil of *P. cablin* against *K. oxytoca*. The methodology used investigates the Minimum Inhibitory Adherence Concentration (MIAC) of the oil, which was determined in the presence of 5% sucrose, using dilutions corresponding to the pure essential oil until dilution 1:1024. The MIAC was considered the lowest concentration of essential oil capable of preventing the adherence of *P. cablin* biofilm formed on the glass tube. Thus, the results obtained showed that both the essential oil and the 0.12% chlorhexidine digluconate did not present a MIAC value. It was concluded that the essential oil of *Pogostemon cablin* does not show anti-adherent activity against the clinical strain of *Klebsiella oxytoca* in the tested concentrations.

INTRODUCTION

Hospital infection is characterized as a nonsocial problem in the global sphere, which is linked to a variety of health challenges, such as increasing morbidity, mortality, highly costly hospital stays, and the spread of antimicrobial-resistant bacteria [1,2]. The great majority of these infections are related to biofilms in implanted and invasive medical devices [3,4], and although there is currently a great technological development, all are susceptible to the risk of microbial colonization and infection [4,5].

Taking this into consideration, *Klebsiella* species stand out, an important human pathogen that is directly related to the increase in morbidity among the population of many patients. Among the species studied, *Klebsiella oxytoca* has been isolated from different clinical samples, mainly from blood and respiratory secretions, and its presence may be related to the weakened immunological condition of the patient [6]. This bacterium is able to form biofilms in most environments, as an opportunistic pathogen that colonizes hospital devices [7].

These biofilms consist of one or more communities of microorganisms, embedded in a matrix, adhered to each other and/or to surfaces or interfaces[8,9]. In which the exopolysaccharide matrix, which is secreted to the external environment, acts as a physical barrier not allowing antimicrobial agents to enter this organization, especially those hydrophilic and positively charged. [10]. For this reason, the organization of these bacteria in biofilms makes their elimination more difficult.

Preventive and innovative strategies should be developed for prevention in the stages of biofilm formation, because the treatment of the mature biofilm is much more difficult and complex. Therefore, several extracts and essential oils have been researched, because natural medicines have good popular acceptance in Brazil and present good market prospects. Among them, there are the essential oils, which have several pharmacological properties [11,12,13].

The essential oil of *Pogostemon cablin* that belongs to the Lamiaceae family [14] has been the target of many researches due to its great phytochemical potential. The literature points out that this species has wide pharmacological activity, among them: anti-emetic, antitrypanosomal, insecticidal [15], antimicrobial, besides being highly used within aromatherapy [14].

Based on information about the therapeutic potential of essential oils and the importance of combating infections caused by multidrug-resistant bacteria, this innovative work seeks to evaluate the possible anti-adherent activity in order to unveil the mechanism of action of *Pogostemon cablin* essential oil against *Klebsiella oxytoca*.

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

Pogostemon cablin essential oil was purchased from Quinari® Industry (Ponta Grossa - PR). For the pharmacological assays, the substance was solubilized in DMSO and tween 80, as well as diluted in distilled water. The concentration of DMSO (dimethylsulfoxide) used was less than 0.1% v/v. The project followed the rules of CGEN - Genetic Heritage Management Council, registered in the SISGEN platform under protocol number A5FA9A1.

Microorganisms

A clinical strain of *Klebsiella oxytoca* (Ko 01) was used and maintained on Muller-Hinton (MH) Agar at 4°C. The inoculum was obtained from overnight cultures on MH at 35°C - 37°C and diluted in sterile saline to obtain a final concentration of approximately 1.5 x 10^8 colony forming units per mL (CFU/mL), adjusted for turbidity by comparing with the 0.5 tube of the McFarland scale [16].

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 MIAC (Minimum Inhibitory Adherence Concentration)

The Minimum Inhibitory Adherence Concentration (MIAC) of *Pogostemon cablin* essential oil was determined in the presence of 5% sucrose, according to Albuquerque et al (2010) with modifications, using dilutions corresponding to the compound up to 1:1024 dilution. From the bacterial growth, the clinical strain of *Klebsiella oxytoca* was grown at 35ºC - 37 ºC in Mueller Hinton broth (DIFCO, Michigan, 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. Incubation was done at 35ºC - 37ºC 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 assay was performed in duplicate. The same procedure was performed for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA). MIAC was considered the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube [17].

RESULTS AND DISCUSSION

The results found about the Minimum Inhibitory Adherence Concentration (MIAC) of *Pogostemon cablin* essential oil against the clinical strain of *Klebsiella oxytoca* are shown in Table 1. After analyzing the results, it was observed that both *Pogostemon cablin* essential oil and 0.12% chlorhexidine digluconate did not show a MIAC value.

Table 1. Minimum Inhibitory Adherence Concentration (MIAC) in µg/mL of *Pogostemon cablin* essential oil and 0.12% Chlorhexidine digluconate against clinical strain of *K. oxytoca*.										
***Pogostemon cablin* 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
	-	-	-	-	-	-	-	-	-	-

The presence of adhesive structures allows the bacteria to colonize the host and produce a biofilm and the restriction of antibiotic penetration into the cells. Although there is not a large body of literature on biofilm formation among *K. oxytoca* isolates, in the work of Shrief and colleagues [18] (2022), biofilm formation among *K. oxytoca* strains was found to be relatively high, where 40% of isolates tended to form a biofilm [18].

Bilcu and colleagues [19] (2014) sought to combine the properties of magnetic nanoparticles with the antimicrobial activity of three essential oils, these being Vanilla, Patchouli and Ylang Ylang, to obtain new nanobiosystems that could be used as coatings for hospital materials, such as catheters, which have a higher resistance to *Staphylococcus aureus* and *Klebsiella pneumoniae* due to the adherence of these clinical strains to the tubes forming a biofilm.

The results showed that patchouli and ylang-ylang essential oils mainly inhibited the initial adhesion phase of S. aureus biofilm development. In the case of K. pneumoniae, all tested nanosystems showed similar efficiency, being active mainly against K. pneumoniae adhesion for the tested catheter specimens [19].

Again, in the work of Kalra and co-workers [20] (2006) patchouli oil showed its anti-adherent potential. It was effective in preventing biofilm formation by foodborne pathogens, *Staphylococcus aureus*.

P. cablin Benth or Patchouli, as it is popularly known, is an important aromatic plant native to Southeast Asia and is currently widespread among tropical and subtropical regions, being cultivated in many countries, including China, Indonesia, the Philippines and Thailand [21]; This species is a dicotyledonous plant, with an erect and branched structure, elongated, with oval leaves and abaxial trichomes. Its inflorescence can be terminal or axillary, dense, with small, irregular, bisexual, white to purple flowers and its size varies from 0.5m to 1.0m [22]. It has numerous pharmacological properties that are extremely beneficial, among them are antimicrobial, antioxidant, analgesic, anti-inflammatory, antiplatelet, antithrombotic, aphrodisiac, antidepressant, antimutagenic, antiemetic, fibrinolytic, and cytotoxic activity [23].

All this pharmacological activity is due to the components, present in several classes of secondary metabolites, including essential oils. According to Santos and collaborators [22] (2022), the main components are patchouli alcohol, pogostone, α-guaiene, δ-guaiene, β-caryophyllene, transcaryophyllene, α-patchoulene, β-patchoulene, and β-elemene. Sesquiterpenes are the majority constituents and thus guarantee most of the pharmacological activities attributed to the plant, among them patchouli alcohol.

CONCLUSION

Concerning the anti-adherent effect of *Pogostemon cablin* essential oil, new tests are necessary, for although it has not shown any effect with the methodology used, its therapeutic potential against other microorganisms should not be disregarded, or even its association to conventional drugs in order to potentiate its effect.

REFERENCES

1. Medeiros EAS, Rosenthal C. A prática segura e a qualidade na atenção In: O controle da infecção hospitalar no Estado de São Paulo. São Paulo: Conselho Regional de Medicina do Estado de São Paulo, 2010. [Acesso em 17 fev. 2016] Disponível em: http:/ /www.mpsp.mp.br/portal/page/portal/Saude_Publica/ infeccao_hospitalar_2010.pdf
2. Bordignon JC, Lima LR. Etiologia de infecções hospitalares e perfil de sensibilidade aos antimicrobianos em um hospital do sudoeste do Paraná, Brasil. RBAC. 2017;49(3):283-8.
3. Otto, M. Staphylococcal Infections: Mechanisms of Biofilm, Maturation and Detachment as Critical Determinants of Pathogenicity. Annu Rev Med.v. 2013; 64(1):1–14.
4. Marson PG, Nepomuceno VR, Eugenio F, Neves TN, da Silva MG, Soares MP, Mundim AP. Associação entre biofilme bucal e aspirado traqueal em pacientes com pneumonia associada à ventilação mecânica. Rev Cereus. 2020;12(3):272-288.

5. Busscher HJ, Van Der Mei HC, Subbiahdoss G, Jutte PC, Van Den Dungen JJ, Zaat SA, Schultz MJ, Grainger DW. Biomaterial associated infection: locating the finish line in the race for the surface. Sci. Transl. Med. 2012; 4(1):1-11.
6. Singh L, Cariappa MP, Kaur M. *Klebsiella oxytoca*: An emerging pathogen?. MJAFI. 2016;72(1):59-61.
7. Ghasemian A, Mobarez AM, Peerayeh SN, Abadi AB. The association of surface adhesin genes and the biofilm formation among *Klebsiella oxytoca* clinical isolates. New microbes and new infections. 2019;27(1):36-39.
8. Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ. Bacterial biofilms in nature and disease. Annu. Rev. Microbiol. 1987;41(1):435-464.
9. Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilmes: uma forma emergente de vida bacteriana. Nat. Rev. Microbiol. 2016;14(9):563-575.
10. Rama, D., Fonseca, B., & Blanck, M. (2018). 1a Recomendação brasileira para o gerenciamento de biofilme em feridas crônicas e complexas.
11. Francisco KSF. Fitoterapia: uma opção para o tratamento odontológico. Revista Saúde. 2010;4(1):18-24.
12. Cechinel Filho V. Principais avanços e perspectivas na área de produtos naturais ativos: estudos desenvolvidos no NIQFAR/UNIVALI. Quím. Nova. 2000;23(5):680-685.
13. Aleluia CM, Procópio VC, Oliveira MTG, Furtado PGS, Giovannini JFG, Mendonça SMS. Fitoterápicos na odontologia. Revista de odontologia da UNICID. 2017;27(2):126-134.
14. Arrigoni-Blank MDF, Santos PS, Blank AF, Brito FDA, Andrade TM, Nizio DADC, Alves PB. Influence of storage temperatures and storage time of dry leaves on patchouli [*Pogostemon cablin* (Blanco) Benth.] essential oil. Biosci. j.(Online). 2020;36(4):1175-1184.
15. Pimenta E, da Cruz R, Diniz-Neto H, Silva D, de Oliveira H, Buccini D, Lima E, de Oliveira Filho A. Avaliação da atividade antifúngica do óleo essencial de *Pogostemon cablin* (Blanco) Benth. (Lamiaceae) contra cepas de Candida glabrata. Sci. plena,. 2019;15(6).
16. Bona EAMD, Pinto FGDS, Fruet TK, Jorge TCM, Moura ACD. 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;81(3):218-225.
17. Albuquerque ACL, Pereira MDSV, Pereira JV, Pereira LF, Silva DF, MacedoCosta MR. Efeito antiaderente do extrato da Matricaria recutita Linn. Sobre microorganismos do biofilme dental. Rev Odontol UNESP. 2010;39(1):21-5.
18. Shrief, R., Hassan, R. H., Zaki, M. E. S., & Rizk, M. A. Molecular Study of *Klebsiella Oxytoca* Associated with Urinary Tract Infection in Children. Open Microbiol. J. 2022;16(1).
19. Bilcu M, Grumezescu AM, Oprea AE, Popescu RC, Mogoșanu GD, Hristu R, Stanciu GA, Mihailescu DF, Lázar V, Bezirtzoglou E, Chifiriuc MC. Efficiency of vanilla, patchouli and ylang ylang essential oils stabilized by iron oxide@ C14 nanostructures against bacterial adherence and biofilms formed by Staphylococcus aureus and Klebsiella pneumoniae clinical strains. Molecules. 2014;19(11):17943-17956.
20. Kalra A, Prakasa Rao EVS, Khanuja SPS. Tecnologias de cultivo e processamento de patchouli (*Pogostemon cablin*). J. Med. Arom. Plants Sei. 2006;28(1) :414-419.
21. Junren C, Xiaofang X, Mengting L, Qiuyun X, Gangmin L, Huiqiong Z, Guanru C, Xin X, Yanpeng Y, Fu P, Cheng P. Pharmacological activities and mechanisms of action of *Pogostemon cablin* Benth: a review. Chin Med. dezembro de 2021;16(1):5.
22. Santos LL, Brandão LB, da Costa ALP, Martins RL, Rodrigues ABL, Lobato AA, de Almeida SMS. Bioinsecticidal and Pharmacological Activities of the Essential Oil of *Pogostemon cablin* Benth Leaves: A Review. Pharmacogn Rev. 4 de agosto de 2022;16(32):139–45.
23. Ribeiro AS, Bertolucci SKV, Carvalho AA de, Tostes WN, Coelho AD, Pinto JEBP. Light intensities alter growth and essential oil of patchouli under shade nets. Cienc Rural. 2022;52(5):e20210118.