Brazilian Journal of Biology®

ISSN 1519-6984 (Print)
ISSN 1678-4375 (Online)

Original Article

Evaluation of the antimicrobial effect of the *Origanum vulgare L* essential oil on strains of *Klebsiella pneumoniae*

Avaliação do efeito antimicrobiano do óleo essencial de *Origanum vulgare L.* sobre cepas de *Klebsiella pneumoniae*

S. L. Silva[a*], F. S. M. Araújo[b], P. O. A. Silva[b], E. V. A. Silva[b], M. M. S. L. Bezerra[b], A. F. Diniz[c], D. M. Oliveira[d], H. O. Jesus[d], B. B. Nascimento-Junior[d], L. A. D. M. Medeiros[b] and A. A. Oliveira-Filho[a]

[a]Universidade Federal da Paraíba – UFPB, Programa de Pós-graduação em Desenvolvimento e Inovação Tecnológica em Medicamentos – PPgDITM, João Pessoa, PB, Brasil

[b]Universidade Federal de Campina Grande – UFCG, Centro de Saúde e Tecnologia Rural, Departamento de Odontologia, Patos, PB, Brasil

[c]Universidade Federal de Campina Grande-UFCG, Centro de Saúde e Tecnologia Rural, Programa de Pós-graduação em Ciência e Saúde Animal, Patos, PB, Brasil

[d]Universidade Estadual do Sudoeste da Bahia – UESB, Programa de Pós-graduação em Química – PGQUIM, Jequié, BA, Brasil

Abstract

Bacteria may be the initial cause of certain pathologies as well as a secondary agent responsible for the development of complications such as pressure ulcer infections. Pressure ulcers are a persistent health problem, especially in immunocompromised patients, and associated with infection by opportunistic microorganisms with antimicrobial resistance, such as *Klebsiella pneumoniae*, highlight the need for the development of new antimicrobial approaches. Thus, the aim of this study was to evaluate the antibacterial and anti-adherent activity of *Origanum vulgare L.* (oregano) essential oil against *Klebsiella pneumoniae* strains, as well as the effect of its association with synthetic antimicrobials. To this end, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) analyses were performed on microdilution plates. The assay of the Minimum Inhibitory Adherence Concentration (MIAC), with test tubes. As well as, the association study through the infusion disc method containing ampicillin (AMP), gentamicin (GEN), ciprofloxacin (CIP) and ceftriaxone (CEF). Therefore, it was possible to obtain that the essential oil of oregano presents antimicrobial and bactericidal activity, with MIC ranging between 128µg/mL and 256 µg/mL and MBC between 256 µg/mL and 512 µg/mL, on the tested *K. pneumoniae* strains. When used in association with ampicillin and gentamicin, oregano essential oil showed synergistic effect for some strains. Therefore, it is observed that the tested essential oil can act as a promising antibacterial in the treatment of diseases caused by *K. pneumoniae.*

Keywords: antibacterial activity, antimicrobial agents, microbiology, *Origanum vulgare L*, *Klebsiella pneumoniae*.

Resumo

As bactérias podem ser a causa inicial de determinadas patologias como também um agente secundário responsável pelo desenvolvimento de complicações como as infecções das lesões por pressão. As lesões por pressão configuram um problema de saúde persistente, principalmente em pacientes imunocomprometidos, e associadas a infecção por microrganismos oportunistas com resistência antimicrobiana, como por exemplo a *Klebisiella pneumoniae*, ressaltam a necessidade do desenvolvimento de novas abordagens antimicrobianas. Assim, o objetivo deste estudo foi avaliar a atividade antibacteriana e antiaderente do óleo essencial de *Origanum vulgare* L. (orégano) contra cepas da *Klebisiella pneumoniae,* bem como o efeito da sua associação com antimicrobianos sintéticos. Para isso foram realizadas as análises da Concentração Mínima Inibitória (CIM) e Concentração Bactericida Mínima (CBM) em placas de micro diluição. O ensaio da Concentração Mínima de Aderência (CIMA), com tubos de ensaio. Como também, o estudo de associação através do método de disco de infusão contendo ampicilina, gentamicina, ciprofloxacino e ceftriaxona. Assim sendo, foi possível obter que o óleo essencial de orégano apresenta atividade antimicrobiana e bactericida, com CIM variando entre 128µg/mL e 256 µg/mL e CBM entre 256 µg/mL e 512 µg/mL, sobre as cepas de *K. pneumoniae* testadas. Quando utilizado em associação com a ampicilina e gentamicina, o óleo essencial de orégano demonstrou efeito sinérgico para algumas cepas. Portanto, observa-se que o óleo essencial testado pode atuar como um promissor antibacteriano no tratamento de doenças causadas por *K. pneumoniae*.

Palavras-chave: atividade antibacteriana, antimicrobianos, microbiologia, *Origanum vulgare* L, *Klebsiella pneumoniae*.

*e-mail: sonalydelima@gmail.com
Received: November 7, 2022 – Accepted: December 30, 2022

1. Introduction

Bacteria are causative of quite simple and even more complex diseases. They present association and are responsible for several disease states, such as urinary tract infections, gastrointestinal diseases, infections: respiratory tract, central nervous system, bones and joints; skin conditions, cursing with acute and chronic conditions as, for example, the chronicity of pressure injury healing, resulting from bacterial invasion and biofilm formation (Baron et al., 2013; Freire et al., 2013; Hannigan et al., 2018; Paula et al., 2020).

In this sense, pressure ulcers (PUs) characterized by discontinuity and interruption of the normal skin structure, by pressure or shear force, present themselves as an open lesion, without protective barrier predisposed to immediate contamination, and can be colonized by the host microflora or by microbes of the environment, exposing an ideal environment for proliferation of pathogenic microorganisms, which after their entry develop and originate infectious processes, such as *Klebsiella pneumoniae* in ICU environment (Martins, 2010; Coates et al., 2014; Cardoso, 2015; Wert et al., 2020).

In turn, *Klebsiella pneumoniae* (Kp) is a Gram-negative bacillus, from the Enterobacteriaceae family, facultative aerobic, but with better growth in aerobic conditions. Its colonization in humans probably occurs through contact with various environmental sources and can be found colonizing the oropharynx and feces of healthy people, or as an opportunist in the body of immunocompromised people or with open wounds and scars, leading to infection (Santos, 2007; Almeida, 2013; Seibert et al., 2014).

Moreover, it stands out for being a producer of an enzyme called carbapenemase, and therefore also receives the acronym KPC - *Klebsiella pneumoniae* carbapenemase. Therefore, it is resistant to antibiotics of the carbapenem class: Meropenem, Ertapenem, and Imipenem, besides being able to inactivate β-lactam agents, such as cephalosporins, penicillins, and monobactams. In addition, it is known that KPC presents a high potential for dissemination, being *Klebsiella pneumoniae* a bacterium with a high capacity to transfer its genetic material, and consequently, its resistance genes, which is associated with the difficulty of infection control involving this bacterium (Cunha, 2014; Nirwati et al., 2019).

Therefore, the main complication of PUs is infection, which associated with risk factors such as increased age, unconsciousness, urinary and/or intestinal incontinence, poor nutritional status, diabetes mellitus, paralysis and cardiovascular diseases determine the morbidity and mortality of the pathology (Luz et al., 2010; Wert et al., 2020).

Soon PUs are one of the current concerns in hospitals and patient care services, because antimicrobial resistance hinders therapy and consequently increases the risk of morbidity and mortality (Mariano and Berreta, 2019).

Given this scenario, a need for the development of new pharmacological therapies for antibacterial treatment with low or no resistance is recognized. Thus, there are studies exploring the use of herbal medicinal compounds such as curcumin from saffron, aloesin from aloe vera, thymol from thyme and others in the treatment of PUs (Aburayan et al., 2020).

Studies show that terpenes are an important category of plant-derived compounds and can interact with each other and with bacterial cells to increase or decrease each other's antibacterial effects. And they observed that eugenol, terpineol, carveol, citronellol, geraniol showed antibacterial activity and linked this effect to the presence of hydroxyl groups (Zengin and Baysal, 2014; Mughal et al., 2024).

Not only, but more specifically, when analyzing the essential oil of oregano, it was possible to observe that oregano is rich in carvacrol and thymol, has as its *Origanum vulgare L.* essential oil (OEO) and presents activity on digestive, respiratory or dermatological disorders, with antibacterial, antifungal, antiparasitic, antioxidant, anti-inflammatory and antitumor effects (Fournomiti et al., 2015; Lombrea et al., 2020).

In this context, this work sought to evaluate the antibacterial and anti-adherent activity and the association study with synthetic antimicrobials and oregano oil against strains of *Klebsiella pneumoniae*, as a promising strategy for management and infection control of pressure sores.

2. Materials and Methods

2.1. In vitro assays

2.2. Test substance

The oregano essential oil (*Origanum vulgare*) was purchased from Industria Quinari (Ponta Grossa- PR). For the pharmacological tests, the substance was solubilized in DMSO and diluted in distilled water. The concentration of DMSO (dimethyl sulfoxide) used was less than 0.1% v/v.

2.3. Analysis of the essential oil by Gas Chromatography Coupled with Mass Spectrometry (GC-MS)

The oregano essential oil was analyzed for its chemical composition using a gas chromatograph coupled to a quadrupole mass spectrometer (GC-MS), Shimadzu model GC-QP2010 (Shimadzu, Kyoto, Japan), using a DB-5 fused silica capillary column (30m x 0.25mm i.d. x 0.25µm). The chromatographic conditions used were: injector at 220 °C and split ratio 1:10 (3.0 min), He carrier gas at 0.6 mL/min, interface temperature at 250 °C and electron ionization source (35-350 m/z), oven temperature ramp: 40 °C (2 min), with increment of 3 °C/min up to 240 °C for 5 min and injected volume of 1 L (1% solution in dichloromethane).

The identification of the compounds was performed by comparing their fragmentation patterns with the mass spectra from the NIST 14 database (NIST/EPA/NIH Mass Spectral Library, 2014) present in the GC-MS; also, by comparing these with literature data (Adams, 2007), and through their linear retention indices (LRIs), calculated in relation to the retention times of a homologous series of hydrocarbons (C8 to C26) injected under the same conditions as the sample. The linear retention index was based on Van den Dool and Kratz (1963) and Adams (2007). For identification by comparison with spectra available in these databases only matches above 90% were considered.

2.4. Microorganisms

Klebsiella pneumoniae strains (ATCC 13883, Kp 101, Kp 103, Kp 104 Kp 105 and Kp 110) were used. All strains were maintained on Muller Hinton Agar (MHAG) at 4 °C. The inoculate were obtained from overnight cultures in MH at 37 °C and diluted in sterile saline to obtain a final concentration of approximately 1.5 x 108 colony forming units per mL (CFU/mL), adjusted by turbidity compared to the 0.5 tube of the McFarland scale (Bona et al., 2014).

2.5. Culture medium

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

2.6. 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. 100µL of double concentrated Mueller Hinton broth and 100µL of oregano essential oil at the initial concentration of 2048 µg/ml were added, were dispensed into the wells of the first row of the plate. And by serial dilution in ratio of two, the concentrations of 1024, 512, 256, 128, 64, 32, 16, 8 and 4 µg/ml were obtained, so that in the first row of the plate is the highest concentration and in the last, the lowest concentration.

The determination of the MIC was conducted with 10µL of the microorganism in each cavity, approximately 1.5 x 108 CFU/mL A sterility control was also prepared in the penultimate well, with only 200µL of the broth, and in the last well the growth control was performed, containing 100µL of the double concentrated Muller Hinton broth and the microorganism suspension, and the entire assay was performed in duplicate. The plates were incubated at 35±2 ºC for 24 hours, after the incubation time, the first reading of the results was performed. Then, 20µL of sodium resazurin solution (SIGMA) were added, previously solubilized in sterile distilled water at a concentration of 0.01% (w/v), recognized as a colorimetric indicator of oxide-reduction for bacteria, then another incubation was performed at 35±2 ºC.

The reading was done, visually, by the absence or presence of growth of the microorganism due to the formation of a cluster of cells (button) and also by the observation of the change in color of the solution, from blue to pink, indicating its growth. The MIC was determined as the lowest concentration of the product that inhibited the visible growth of the microorganism assayed, verified by a no change in the coloration of the indicator dye (Palomino et al., 2002; Ostrosky et al., 2008; CLSI, 2012; Bona et al., 2014).

2.7. Determination of the Minimum Bactericidal Concentration (MBC)

The minimum bactericidal concentration (MBC) of oregano essential oil was also determined for *Klebsiella pneumoniae* strains. After the MIC reading, inoculations (10µL) of up to three dilutions prior to the MIC value were made into Mueller-Hinton broth medium (100µL/cavity) in sterile microdilution plate, then passed for incubation at 35±2 °C for 24 hours, after which time 20µL of resazurin was added. The assays were incubated at 35±2 ºC for another 24 hours for confirmation of the concentration capable of inhibiting the total growth of the bacterial species, verified by a non-change in the coloration of the indicator dye (Ncube et al., 2008; Guerra et al., 2012).

2.8. Determination of the MIAC

The Minimum Inhibitory Adherence Concentration (MIAC) of oregano essential oil was determined in the presence of 5% sucrose, according to Albuquerque et al. (2010) with modifications, using concentrations corresponding to the pure oil up to 1:1024 dilution. From the bacterial growth, the strain of *Klebsiella pneumoniae* (KP 105) was grown at 35±2 ºC in Mueller Hinton broth (DIFCO, Michigan, United States), subsequently 0.9mL of the subculture was distributed in test tubes and then 0.1mL of the solution corresponding to the compound dilutions was added. Incubation was performed at 35±2 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 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). The MIAC was considered the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube.

2.9. Study of the association of oregano essential oil with synthetic antimicrobial agents

For the association study of oregano essential oil with antimicrobials, it was carried out by means of the disc diffusion technique in solid medium using filter paper discs

(Ampicillin- AMP, Gentamicin- GEN, Ciprofloxacin- CIP and Ceftriaxone- CEF). In smooth sterile Petri plates containing Muller Hinton agar medium previously inoculated with the bacteria suspension, discs containing antimicrobials were introduced and 20µL of the MIC of the oregano essential oil were added, then the plates were incubated at 35±2 ºC for 24-48h, followed by their reading. It was considered as synergistic effect, when the microbial growth inhibition halo formed by the association (oil + antimicrobial) had a diameter ≥ than 2mm, when compared to the inhibition halo formed by the action of the isolated antimicrobial, When the formation of the halo of inhibition resulting from the association had a diameter smaller than the one developed by the isolated action of the antimicrobial, it was considered an antagonistic effect, and when the halo of inhibition resulting from the association had a diameter equal to the one resulting from the isolated application of the antimicrobial, it was considered an indifferent effect (Cleeland and Squires, 1991). All tests were performed in duplicate.

3. Results and Discussion

Infectious diseases are the second leading cause of death in the world, accounting for approximately 15 million deaths each year. This high mortality rate is mainly due to antibiotic resistance. For this reason, natural products have been evaluated as a source of new pharmacological molecules with antimicrobial potential (Aelenei et al., 2016).

In this sense, several essential oils have been evaluated and, in this research, the oregano essential oil was analyzed for its chemical constituents through the retention time presented in each peak of the spectrum, as can be seen in Table 1.

As a result, we identified a total of 9 constituents present in oregano essential oil: alpha-pinene, camphene, myrcene, carene, cymene, gamma-terpinene, linalool, carvacrol and caryophyllene. And from the analysis of the percentages, we verified that carvacrol presents itself as the majority compound, with a high percentage (40.52%).

Corroborating what was described by Penteado et al. (2021), who, in their analyses, found carvacrol also as one of the major components of the oregano essential oil, with a percentage of 69.1%, being this oil also evaluated by gas chromatography coupled to mass spectrometry (GC-MS). Similarly, Gomes et al. (2019), using gas chromatography coupled to mass spectrometry when analyzing the chemical composition of OEO obtained a percentage of 45.74% of carvacrol, and further noted that this is the majority compound of the oil. These variations in percentages are explained from environmental conditions such as altitude and water availability, season, phenological stage of the plant, the type of extraction, among other factors (Lombrea et al., 2020).

The carvacrol as a component of the OEO presents itself as the main compound responsible for the antibacterial activity (Oliveira et al., 2008). Ultee et al. (2002) describes that the carvacrol together with the thymol (present in thyme essential oil), are able to disintegrate the external membrane of gram-negative bacteria. And, more specifically, carvacrol is responsible for depleting the intracellular ATP (adenosine triphosphate) reserve and increasing the permeability of the cytoplasmic membrane for the entry of cations, altering essential cellular processes, resulting in bacterial death (Magi et al., 2015).

Therefore, in the present research, the Minimum Inhibitory Concentration (MIC) of oregano essential oil was evaluated, in liquid medium and at the different concentrations suggested in the methodology. Then, the MIC was determined by the lowest concentration capable of visibly inhibiting bacterial growth.

As shown in Table 2, it was observed that the MIC results for oregano essential oil was 128 µg/mL for three of the six strains of *Klebsiella pneumoniae* tested. Thus, a MIC50 (lowest concentration capable of inhibiting growth by 50%) of 128µg/mL was considered.

According to Sartoratto et al. (2004), the antimicrobial activity can be classified as strong when it has MIC up to 500µg/mL, moderate for MIC from 600µg/mL to 1500µg/mL and weak for MIC above 1500µg/mL. Therefore, the present study, has oregano essential oil showing strong antimicrobial activity on *Klebsiella pneumoniae* strains.

An additional analysis refers to the bactericidal or bacteriostatic characteristic of the tested product. For this, it is necessary to determine the Minimum Bactericidal Concentration (MBC) of oregano EO on *Klebsiella pneumoniae* strains. Thus, the MBC was determined from the lowest concentration of the oil that resulted in visible inhibition of the growth of the microorganism. According to Table 3, it is observed that the values obtained were the same for some strains and higher for others in relation to the MIC.

Finally, for determination of bacteriostatic or bactericidal activity, the ratio of MBC and MIC is then analyzed. For, according to Hafidh et al. (2011), the compound is considered bactericidal when this ratio is between 1: 1 to 2: 1, and the compound is considered as bacteriostatic when the ratio is greater than 2:1. Therefore, in the present study, oregano EO has bactericidal activity, with MBC ranging between 256 µg/mL and 512 µg/mL and MIC between 128 µg/mL and 256 µg/mL against *K. pneumoniae* strains.

Corroborating this data, it is possible to observe in the literature, studies such as that of Kozics et al. (2019), in which they obtained that the essential oil of oregano showed an MIC of 0.05% m/v and MBC of 0.05%m/v on *K. pneumoniae* KMB522 bacterial strains, through the

Table 1. Chemical composition of oregano essential oil.

Peaks	Tr	Compound	LIR_{Lit}	LIR_{Exp}	Area (%)
1	8.586	Alpha-Pineno	928	939	3.73
2	9.234	Camphene	944	953	1.07
3	11.090	Myrcene	988	987	3.57
4	12.244	Carene	995	997	1.55
5	12.607	Cimene	1024	1026	23.39
6	14.199	Gamma-terpinene	1054	1058	8.23
7	16.243	Linalool	1095	1098	1.79
8	25.623	Carvacrol	1302	1298	40.52
9	30.505	Caryophyllene	1413	1404	4.37

(Tr) Time retention; (LIR_{Lit}) Linear Retention Rate from literature; (LIR_{Exp}) Experimental Linear Retention Rate.
Source: Research data (2022).

Table 2. Minimum inhibitory concentration (MIC) of oregano essential oil against different strains of *Klebsiella pneumoniae.*

Bacterial strain/ Substance	*Atcc 13883*	*Kp101*	*Kp103*	*Kp104*	*Kp105*	*Kp110*
1024 µg/mL	-	-	-	-	-	-
512 µg/mL	-	-	-	-	-	-
256 µg/mL	-	-	-	-	-	-
128µg/mL	-	+	-	+	+	-
64 µg/mL	+	+	+	+	+	+
32µg/mL	+	+	+	+	+	+
16µg/mL	+	+	+	+	+	+
8µg/mL	+	+	+	+	+	+
4µg/mL	+	+	+	+	+	+
Sterility control	-	-	-	-	-	-
Growth control	+	+	+	+	+	+

(+) With growth; (-) no growth. Source: research data (2022).

Table 3. Minimum bactericidal concentration (MBC) of oregano essential oil against different strains of *Klebsiella pneumoniae.*

Bacterial strain/ Substance	*Atcc 13883*	*Kp101*	*Kp103*	*Kp104*	*Kp105*	*Kp110*
1024 µg/mL	-	-	-	-	-	-
512 µg/mL	-	-	-	-	-	-
256 µg/mL	-	+	-	-	-	-
128 µg/mL	+	+	+	+	+	+
Sterility control	-	-	-	-	-	-
Growth control	+	+	+	+	+	+

(+) With growth; (-) no growth.
Source: research data (2022).

microwell dilution method. Thus, the authors considered oregano essential oil bactericidal, thus effective against *K. pneumoniae*.

Kosakowska et al. (2021), on the other hand, evaluated the essential oil of *Oregano vulgare (Greek)* on several gram-positive and gram-negative bacterial strains, by the microdilution method. And, they obtained an MIC ranging between 2-8 mg × mL-1 and MBC between 4-16 mg × mL-1 showing the two strains of E. coli ATCC 25922 and micro-well. coli O157:H7ATCC 700728, both gram negative, as the most susceptible strains among the tested strains. That said, they concluded that *Oregano vulgare (Greek)* showed bacteriostatic and bactericidal activity.

From the point of view of the compounds responsible for the main therapeutic activities identified in the essential oil of oregano, it is observed as more representative the carvacrol and thymol (Bora et al., 2022). Thus, it is verified in the study of Guarda et al. (2011), that the antimicrobial capacity of the microencapsulated carvacrol and thymol was 0.225mg/mL and 0.250 mg/mL respectively, against the microorganisms of *Escherichia coli, Staphylococcus aureus, Listeria innocua, Saccharomyces cerevisiae and Aspergillus niger.*

In another study, analyzing the activity of oregano essential oil, with carvacrol as the main constituent, on bacteria, fungi and yeast, and by the well microdilution method, it was obtained that bacteria were the most affected, and against these bacterial strains, an MIC between 31.25-128 µl/mL was identified (Jan et al., 2020).

And also, in the study by Fratini et al. (2017), when analyzing the minimum inhibitory concentration of oregano essential oil, it was identified that its main constituent was carvacrol (65.93%), and it was obtained from the microdilution assay a MIC ≤ 0.240 mg/mL for eight strains of *S. aureus* and MIC ≤ 0.480 mg/mL for another five strains also of *S. aureus*.

Considering the anti-adherent activity, in the present study, it was observed that both oregano essential oil and chlorhexidine digluconate 0.12% failed to inhibit biofilm formation at the tested proportions, as shown in Table 4.

And reinforcing this result, Zapién-Chavarría et al. (2019), when analyzing the susceptibility of uropathogenic strains (biofilm-forming and resistant to multiple conventional drugs), to oregano essential oil of Mexican origin, through the glass tube and microplate tests, found bactericidal activity, and absence of bacteriostatic activity.

However, Lee et al. (2017), analyzed the antibiofilm activity of oregano and red thyme essential oil on uropathogenic *Escherichia coli* strains by confocal laser scanning microscopy. Then, they obtained that the two oils inhibited biofilm formation in more than 60% of the evaluated strains and associated this effect with inhibition of fimbria production and swimming motility.

Table 4. Minimum adherence inhibitory concentration in µg/mL of oregano essential oil and 0.12% chlorhexidine digluconate against *Klebsiella pneumoniae* strain (Kp105).

Oregano Essential Oil								
µg/mL	**1:1**	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**
	+	+	+	+	+	+	+	+
Chlorhexidine digluconate 0.12%								
µg/mL	**1:1**	**1:2**	**1:4**	**1:8**	**1:16**	**1:32**	**1:64**	**1:128**
	+	+	+	+	+	+	+	+

(+) with adhesion to tube wall.
Source: research data (2022).

While, Čabarkapa et al. (2019), aiming to determine the bioactive compounds of the EOs of *Origanum heracleoticum, Origanum vulgare, Thymus vulgaris* and *Thymus serpyllum*, and evaluate their antimicrobial and antibiofilm activity against *Salmonella Enteritidis* performed the biomass biofilm formation assay and incubated with the EOs and the bioactive compounds of the EOs. To this end, it obtained for *Salmonella Enteritidis* biofilms, the EO of *O. heracleoticum* showed the highest effect, followed by *O. vulgare, T. vulgaris*, carvacrol and thymol, while the EO of *T. serpyllum* showed the weakest effect regarding prevention of biofilm growth and eradication of pre-formed biofilms.

Moreover, taking into account the bacterial strain, Ramalho et al. (2020), used strains of *Klebsiella pneumoniae* (kp 102), grown in Mueller Hinton broth in test tubes in order to evaluate the Minimum Inhibitory Concentration of Adherence (MIAC) of the essential oils of *Eucalyptus globulus* and *Eucalyptus citriodora*, and as a positive control was employed chlorhexidine digluconate at 0.12%. Thus, when analyzing the results of the anti-adherent activity of the EOs, it was obtained that the EO of *Eucalyptus globulus* showed good efficiency against biofilm formation by *Klebsiella pneumoniae* showing results equivalent to the control chlorhexidine digluconate 0.12%, and inhibiting biofilm formation at the same concentration 1:8. However, the analysis of the *Eucalyptus citriodora* EO showed biofilm formation to the tube walls, so it did not show inhibition against Klebsiella pneumoniae at any concentration.

And Souza et al. (2021), also using strains of *Klebsiella pneumoniae* (kp 102) grown in Mueller Hinton broth in test tubes, evaluated the anti-adherent activity of the essential oil of *Lavandula hybrid Grosso*, having as positive control the chlorhexidine digluconate at 0.12%. Thus, it was possible to observe that the EO showed a better property than the 0.12% chlorhexidine digluconate, because it presented a lower concentration capable of inhibiting biofilm adherence to the tube, being 1:32 for *Lavandula hybrid Grosso* essential oil and 1:8 for 0.12% chlorhexidine digluconate. Then, they concluded that EO can be used as an alternative method for biofilm control in the presence of *K. pneumoniae.*

Finally, Rajasekharan et al. (2017) investigated the anti-adherent potential of *Arctium lappa Linn* (burdock root) extract and one of its components, chlorogenic acid, on clinical isolates of *Klebsiella pneumoniae*. Burdock root extract (BRE) (20-200 µg/ml) or chlorogenic acid (CGA) (0.028- 0.28 mmol/l) were added to NB medium supplemented with 0.5% glucose and inoculated with 1% bacterial inoculum, and biofilm growth was quantified by biofilm biomass formation assay (crystal violet). Thus, as a result, they obtained that both the extract and the acid, showed significant anti-adherent activity against *K. pneumoniae* strains.

Regarding the association of oregano essential oil with the tested antimicrobials, AMP showed synergistic (50%) and indifferent (50%) effect, synergistic (33%) and indifferent (67%) effect with GEN, and found indifferent (67%), synergistic (16.5%) and antagonistic (16.5%) effect in the presence of CEF. With CIP, an indifferent (50%), synergistic (16.7%), and antagonistic (33.3%) effect was observed. This result was achieved from the comparative observation of the halo of inhibition in the presence of antibiotics, and the halo of inhibition in the presence of the association of antimicrobials with oregano essential oil. This result can be seen in Table 5.

In this case, it is verified in literature researches such as Rosato et al. (2020), in which they evaluated the essential oils of *Cinnammonum zeylanicum, Mentha piperita, Origanum vulgare,* and *Thymus vulgaris*, regarding their anti-biofilm effect and synergism with common antibiotics such as gentamicin, norfloxacin and oxacillin. Then, the association showed a strong destruction of the bacterial biofilm and synergism of action between the EOs and the evaluated antibiotics. Being the interaction of norfloxacin with EOs the most effective among all tested combinations of EO and antibiotic against the strains of *Staphylococcus aureus ATCC 29213, Enterococcus faecalis ATCC 29212, Staphylococcus epidermidis IG4,* and *Staphylococcus aureus IG22*. Corroborating with the present research, from which we can observe that oregano oil showed synergistic effect with ampicillin and gentamicin.

While, in the association study by Uzair et al. (2017), essential oils with the potential to inhibit the in vitro growth of methicillin-resistant *Staphylococcus aureus* (MRSA), isolated and in association with amoxicillin, an antibiotic of the penicillin class, were investigated. To carry out the study, the disk diffusion method was applied to evaluate the activity of ten essential oils extracted from traditional plants: *Thymus vulgaris L, Mentha pulegium, Ocimum sanctum, Mentha piperita, Cymbopogon citratus, Rosmarinus officinalis L., Cortex cinnamom, Citrus nobilis, Citrus deliciosa, Origanum vulgare* and *Mentha sp*. As results, they observed that the essential oils have both bacteriostatic and bactericidal activity higher than that

Table 5. Study of the association of oregano essential oil with synthetic antimicrobials.

Microorganism	Association	AMP	GEN	CEF	CIP
Atcc 13883	HIATB	-	20mm	28mm	12mm
	HIATB + OEO	-	20mm (*)	28mm (*)	30mm (↑)
Kp101	HIATB	-	20mm	26mm	24mm
	HIATB + OEO	-	20mm (*)	26mm (*)	24mm (*)
Kp103	HIATB	-	18mm	26mm	12mm
	HIATB + OEO	-	20mm (↑)	26mm (*)	12mm (*)
Kp104	HIATB	-	16mm	12mm	28mm
	HIATB + OEO	-	20mm (↑)	22mm (↑)	12mm (↓)
Kp105	HIATB	12mm	20mm	24mm	36mm
	HIATB + OEO	18mm (↑)	20mm (*)	24mm (*)	30mm (↓)
Kp110	HIATB	20mm	20mm	28mm	30mm
	HIATB + OEO	20mm (*)	20mm (*)	26mm (↓)	30mm (*)

HIATB: halo of inhibition in the presence of the antibiotic; OEO: oregano essential oil. Source: research data (2022). ↑Synergistic effect. ↓Antagonistic effect. *Indifferent effect.

of synthetic antibiotics when tested in vitro, with higher activity for thyme and cinnamon oil. And oregano and mint (*Mentha pulegium*) showed strong synergistic activity with amoxicillin. The authors attribute this synergism of oregano due to the high carvacrol content in its composition.

And Magi et al. (2015), examined the antibacterial activity of the essential oils of *Origanum vulgare, Thymus vulgaris, Lavandula angustifolia, Mentha piperita* and *Melaleuca alternifolia*, and of carvacrol, against 32 erythromycin-resistant and cell-invasive strains of Group *A Streptococci* (GAS), in isolation, and in association with erythromycin. Overall, they observed that thyme and oregano essential oils demonstrated the greatest antimicrobial activity. And, through quad assays, they obtained a 2 to 2,048-fold reduction in the MIC of erythromycin, with confirmed synergy for carvacrol and erythromycin.

Santana et al. (2020), in order to evaluate the antibacterial activity of the essential oil of *Lavandula hybrid Grosso*, associated with the antimicrobial ampicillin against strains of S. aureus, used the microdilution broth technique to obtain the MIC and then performed the association of essential oil with the antimicrobial ampicillin (10 µg/mL), in disc. As a conclusion, they obtained a MIC of 128 µg/mL of the EO for the strain under analysis and, as for the association test, a synergistic effect of ampicillin with the essential oil of *Lavandula hybrid Grosso* was observed.

In this scenario, it can be observed that essential oils in association with common antimicrobials, may represent alternative strategies in combating bacteria resistant to common isolated antimicrobials (Magi et al., 2015).

4. Conclusion

From the results obtained in this study we can conclude that the essential oil of *Origanum vulgare L.* presents itself as a candidate for the development of new pharmacological therapies for antimicrobial treatment, in order to properly manage pressure lesions infected by *Klebsiella pneumoniae*. Since, OEO showed bactericidal capacity and synergistic effect when used in association with some conventional antimicrobials on strains of this bacterium. However, further studies are needed to confirm and elucidate the mechanisms and patterns of efficiency and efficacy, as well as to develop a suitable pharmaceutical form for the mentioned application.

Acknowledgements

The authors thank the Brazilian agencies "Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq)", "Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)".

References

ABURAYAN, W.S., BOOQ, R.Y., BINSALEH, N.S., ALFASSAM, H.A., BAKR, A.A., BUKHARY, H.A., ALYAMANI, E.J. and TAWFIK, E.A., 2020. The delivery of the novel drug 'Halicin' using electrospun fibers for the treatment of pressure ulcer against pathogenic bacteria. *Pharmaceutics*, vol. 12, no. 12, p. 1189. http://dx.doi.org/10.3390/pharmaceutics12121189. PMid:33302338.

ADAMS, R.P., 2007. *Identification of essential oil components by gas chromatography/mass spectrometry*. 4th ed. Carol Stream: Allured Publishing Corporation.

AELENEI, P., MIRON, A., TRIFAN, A., BUJOR, A., GILLE, E. and APROTOSOAIE, A.C., 2016. Essential oils and their components as modulators of antibiotic activity against gram-negative bacteria. *Medicines*, vol. 3, no. 3, p. 19. http://dx.doi.org/10.3390/medicines3030019. PMid:28930130.

ALBUQUERQUE, A.C.L., PEREIRA, M.D.S.V., PEREIRA, J.V., PEREIRA, L.F., SILVA, D.F., MACEDO-COSTA, M.R. and HIGINO, J.S., 2010. Efeito antiaderente do extrato da *Matricaria recutita* Linn. sobre microorganismos do biofilme dental. *Revista de Odontologia da UNESP*, vol. 39, pp. 21-25.

ALMEIDA, V.V.P., 2013. *Infecções por Klebisiella pneumoniae resistente aos carbapenêmicos em hospital de nível terciário: epidemiologia e caracterização*. Uberlândia: Universidade Federal de Uberlândia. Dissertação de Mestrado em Ciências da Saúde.

BARON, E.J., MILLER, J.M., WEINSTEIN, M.P., RICHTER, S.S., GILLIGAN, P.H., THOMSON JUNIOR, R.B., BOURBEAU, P., CARROLL, K.C., KEHL, S.C., DUNNE, W.M., ROBINSON-DUNN, B., SCHWARTZMAN, J.D., CHAPIN, K.C., SNYDER, J.W., FORBES, B.A., PATEL, R., ROSENBLATT, J.E. and PRITT, B.S., 2013. A guide to utilization of the microbiology laboratory for diagnosis of infectious diseases: 2013 recommendations by the Infectious Diseases Society of America (IDSA) and the American Society for Microbiology (ASM). *Clinical Infectious Diseases*, vol. 57, no. 4, pp. e22-e121. http://dx.doi.org/10.1093/cid/cit278. PMid:23845951.

BONA, E.A.M., PINTO, F.G.S., FRUET, T.K., JORGE, T.C.M. and MOURA, A.C., 2014. Comparação de métodos para avaliação da atividade antimicrobiana e determinação da concentração inibitória mínima (cim) de extratos vegetais aquosos e etanólicos. *Arquivos do Instituto Biológico*, vol. 81, no. 3, pp. 218-225. http://dx.doi.org/10.1590/1808-1657001192012.

BORA, L., AVRAM, S., PAVEL, I.Z., MUNTEAN, D., LIGA, S., BUDA, V., GURGUS, D. and DANCIU, C., 2022. An up-to-date review regarding cutaneous benefits of Origanum vulgare L. essential oil. *Antibiotics*, vol. 11, no. 5, p. 549. http://dx.doi.org/10.3390/antibiotics11050549. PMid:35625193.

ČABARKAPA, I., ČOLOVIĆ, R., ĐURAGIĆ, O., POPOVIĆ, S., KOKIĆ, B., MILANOV, D. and PEZO, L., 2019. Anti-biofilm activities of essential oils rich in carvacrol and thymol against Salmonella Enteritidis. *Biofouling*, vol. 35, no. 3, pp. 361-375. http://dx.doi.org/10.1080/08927014.2019.1610169. PMid:31088182.

CARDOSO, V.M., 2015. *O microbioma humano*. Porto: Faculdade de Ciências da Saúde, Universidade Fernando Pessoa. Doctoral dissertation.

CLEELAND, R. and SQUIRES, E., 1991. Evaluation of new antimicrobials in vitro and in experimental animal infections. Antibiot. *Laboratory Medicine*, vol. 3, pp. 739-787.

CLINICAL AND LABORATORY STANDARDS INSTITUTE – CLSI, 2012. *Approved standards M100-S22. Performance standards for antimicrobial disk susceptibility tests*. 16th ed. Wayne: CLSI.

COATES, R., MORAN, J. and HORSBURGH, M.J., 2014. Staphylococci: colonizers and pathogens of human skin. *Future Microbiology*, vol. 9, no. 1, pp. 75-91. http://dx.doi.org/10.2217/fmb.13.145. PMid:24328382.

CUNHA, V.O., 2014. *Bactérias multirresistentes: Klebsiella pneumoniae carbapenemase enzima KPC nas Infecções Relacionadas à Assistência à Saúde (IRAS)*. Belo Horizonte: Universidade Federal de Minas Gerais, Programa de Pós-graduação em Microbiologia. Undergraduate thesis.

FOURNOMITI, M., KIMBARIS, A., MANTZOURANI, I., PLESSAS, S., THEODORIDOU, I., PAPAEMMANOUIL, V., KAPSIOTIS, I., PANOPOULOU, M., STAVROPOULOU, E., BEZIRTZOGLOU, E.E. and ALEXOPOULOS, A., 2015. Antimicrobial activity of essential oils of cultivated oregano (Origanum vulgare), sage (Salvia officinalis), and thyme (Thymus vulgaris) against clinical isolates of Escherichia coli, Klebsiella oxytoca, and Klebsiella pneumoniae. *Microbial Ecology in Health and Disease*, vol. 26, p. 23289. http://dx.doi.org/10.3402/mehd.v26.23289. PMid:25881620.

FRATINI, F., MANCINI, S., TURCHI, B., FRISCIA, E., PISTELLI, L., GIUSTI, G. and CERRI, D., 2017. A novel interpretation of the Fractional Inhibitory Concentration Index: the case Origanum vulgare L. and Leptospermum scoparium JR et G. Forst essential oils against Staphylococcus aureus strains. *Microbiological Research*, vol. 195, pp. 11-17. http://dx.doi.org/10.1016/j.micres.2016.11.005. PMid:28024521.

FREIRE, I.L.S., ARAÚJO, R.D.O., VASCONCELOS, Q.L.D.A.Q., MENEZES, L.C.C., COSTA, I.K.F. and TORRES, G.D.V., 2013. Perfil microbiológico, de sensibilidade e resistência bacteriana das hemoculturas de unidade de terapia intensiva pediátrica. *Revista de Enfermagem da UFSM*, vol. 3, no. 3, pp. 429-439. http://dx.doi.org/10.5902/217976928980.

GOMES, P.R.B., LISTON, M.S., SILVA, J.P. da, OLIVEIRA, R.W.S. de, LOUZEIRO, H.C., FONTENELE, M.A., PAULA, M., NASCIMENTO, A.R. and MOUCHREK FILHO, V.E., 2019. Estudo da composição química e aplicação do óleo essencial Origanum vulgare L como agente antibacteriano em sururu (Mytella charruana) in natura. *Revista Virtual de Química*, vol. 11, no. 6, pp. 1693-1711. http://dx.doi.org/10.21577/1984-6835.20190119.

GUARDA, A., RUBILAR, J.F., MILTZ, J. and GALOTTO, M.J., 2011. The antimicrobial activity of microencapsulated thymol and carvacrol. *International Journal of Food Microbiology*, vol. 146, no. 2, pp. 144-150. http://dx.doi.org/10.1016/j.ijfoodmicro.2011.02.011. PMid:21411168.

GUERRA, F.Q.S., MENDES, J.M., OLIVEIRA, W., COSTA, J., COUTINHO, H.D.M. and LIMA, E.O., 2012. Chemical composition and antimicrobial activity of Cinnamomum zeylanicum Blume essential oil on multi-drug resistant Acinetobacter spp. strains. *Biofar*, vol. 8, no. 1, pp. 62-70.

HAFIDH, R.R., ABDULAMIR, A.S., VERN, L.S., BAKAR, F.A., ABAS, F., JAHANSHIRI, F. and SEKAWI, Z., 2011. Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. *The Open Microbiology Journal*, vol. 5, no. 1, pp. 96-106. http://dx.doi.org/10.2174/1874285801105010096. PMid:21915230.

HANNIGAN, G.D., DUHAIME, M.B., KOUTRA, D. and SCHLOSS, P.D., 2018. Biogeography and environmental conditions shape bacteriophage-bacteria networks across the human microbiome. *PLoS computational biology*, vol. 14, no. 4, p. e1006099. http://dx.doi.org/10.1371/journal.pcbi.1006099. PMid:29668682.

JAN, S., RASHID, M., ALLAH, E.F.A. and AHMAD, P., 2020. Biological efficacy of essential oils and plant extracts of cultivated and wild ecotypes of Origanum vulgare L. *BioMed Research International*, vol. 2020, p. 8751718. http://dx.doi.org/10.1155/2020/8751718. PMid:32337282.

KOSAKOWSKA, O., WEGLARZ, Z., PIORO-JABRUCKA, E., PRZYBYL, J.L., KRASNIEWSKA, K., GNIEWOSZ, M. and BACZEK, K., 2021. Antioxidant and antibacterial activity of essential oils and hydroethanolic extracts of Greek oregano (O. vulgare L. subsp. hirtum (Link) Ietswaart) and common oregano (O. vulgare L. subsp. vulgare). *Molecules*, vol. 26, no. 4, p. 988. http://dx.doi.org/10.3390/molecules26040988. PMid:33668450.

KOZICS, K., BUCKOVA, M., PUSKAROVA, A., KALASZOVA, V., CABICAROVA, T. and PANGALLO, D., 2019. The effect of ten essential oils on several cutaneous drug-resistant microorganisms and their cyto/genotoxic and antioxidant properties. *Molecules*, vol. 24, no. 24, p. 4570. http://dx.doi.org/10.3390/molecules24244570. PMid:31847159.

LEE, J.-H., KIM, Y.-G. and LEE, J., 2017. Carvacrol-rich oregano oil and thymol-rich thyme red oil inhibit biofilm formation and the virulence of uropathogenic Escherichia coli. *Journal of Applied Microbiology*, vol. 123, no. 6, pp. 1420-1428. http://dx.doi.org/10.1111/jam.13602. PMid:28980415.

LOMBREA, A., ANTAL, D., ARDELEAN, F., AVRAM, S., PAVEL, I.Z., VLAIA, L., MUT, A.M., DIACONEASA, Z., DEHELEAN, C.A., SOICA, C. and DANCIU, C., 2020. A recent insight regarding the phytochemistry and bioactivity of *Origanum vulgare* L. essential oil. *International*

Journal of Molecular Sciences, vol. 21, no. 24, p. 9653. http://dx.doi.org/10.3390/ijms21249653. PMid:33348921.

LUZ, S.R., LOPACINSKI, A.C., FRAGA, R.D. and URBAN, C.D.A., 2010. Úlceras de pressão. *Geriatria & Gerontologia*, vol. 4, no. 1, pp. 36-43.

MAGI, G., MARINI, E. and FACINELLI, B., 2015. Antimicrobial activity of essential oils and carvacrol, and synergy of carvacrol and erythromycin, against clinical, erythromycin-resistant group A Streptococci. *Frontiers in Microbiology*, vol. 6, p. 165. http://dx.doi.org/10.3389/fmicb.2015.00165. PMid:25784902.

MARIANO, G.G. and BERRETA, A.L.R.Z., 2019. Principais microorganismos na lesão por pressão: uma revisão literária. *Revista Científica da Fundação Hermínio Ometto*, vol. 7, no. 1, pp. 94-98.

MARTINS, M.S., 2010. *Infecção em úlceras de pressão e o efeito bactericida dos apósitos com prata*. Porto: Faculdade de Ciências da Saúde, Universidade Fernando Pessoa. Undergraduate thesis.

MUGHAL, T.A., ALI, S., HASSAN, A., KAZMI, S.A.R., SALEEM, M.Z., SHAKIR, H.A., NAZER, S., FAROOQ, M.A., AWAN, M.Z., KHAN, M.A., ANDLEEB, S., MUMTAZ, S., MUMTAZ, S., TAHIR, H.M. and GULZAR, N., 2024. Triagem fitoquímica, atividade antimicrobiana, atividade antioxidante in vitro e in vivo do extrato de casca de raiz de Berberis lycium Royle. *Brazilian Journal of Biology = Revista Brasileira de Biologia*, vol. 84, p. e249742. http://dx.doi.org/10.1590/1519-6984.249742.

NCUBE, N.S., AFOLAYAN, A.J. and OKOH, A.I., 2008. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology*, vol. 7, no. 12, pp. 1797-1806. http://dx.doi.org/10.5897/AJB07.613.

NIRWATI, H., SINANJUNG, K., FAHRUNISSA, F., WIJAYA, F., NAPITUPULU, S., HATI, V.P., HAKIM, M.S., MELIALA, A., AMAN, A.T. and NURYASTUTI, T., 2019. Biofilm formation and antibiotic resistance of Klebsiella pneumoniae isolated from clinical samples in a tertiary care hospital, Klaten, Indonesia. *BMC Proceedings*, vol. 13, suppl. 11, p. 20. http://dx.doi.org/10.1186/s12919-019-0176-7. PMid:31890013.

NIST/EPA/NIH MASS SPECTRAL LIBRARY, 2014. *NIST Standard Reference Database 1A NIST/EPA/NIH Mass Spectral Library (NIST 14) and NIST Mass Spectral Search Program (Version 2.2)*. Gaithersburg: NIST.

OLIVEIRA, D.H., FARIAS, A.M., CLEFF, M.B., MEIRELES, M.C.A. and RODRIGUES, M.R.A., 2008. Caracterização química do óleo essencial de *Origanum vulgare*: análise da relação Timol/Carvacrol. In: *XVII Congresso de Iniciação Científica*, 11-14 November 2007, Pelotas, Brazil. Pelotas, Brazil: Universidade Federal de Pelotas, pp. 1-5.

OSTROSKY, E.A., MIZUMOTO, M.K., LIMA, M.E.L., KANEKO, T.M., NISHIKAWA, S.O. and FREITAS, B.R., 2008. Métodos para avaliação da atividade antimicrobiana e determinação da concentração mínima inibitória (CMI) de plantas medicinais. *Revista Brasileira de Farmacognosia*, vol. 18, no. 2, pp. 301-307. http://dx.doi.org/10.1590/S0102-695X2008000200026.

PALOMINO, J.C., MARTIN, A., CAMACHO, M., GUERRA, H., SWINGS, J. and PORTAELS, F., 2002. Resazurin Microtiter Assay Plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy*, vol. 46, no. 8, pp. 2720-2722. http://dx.doi.org/10.1128/AAC.46.8.2720-2722.2002. PMid:12121966.

PAULA, L.M., GUIMARÃES, A.S., REIS, A.C.R., LIMA, E.G.S., ANDRADE, I.M.D., ROSA, M.N., MARTINS, T.R., ALVES, T.C.L., SARMENTO, V.A. and LUSVARGHI, I.L., 2020. Microbiota intestinal e câncer colorretal: uma revisão bibliográfica. *Revista Eletrônica Acervo Saúde*, vol. 12, no. 11, p. e4672. http://dx.doi.org/10.25248/reas.e4672.2020.

PENTEADO, A.L., ESCHIONATO, R.A., SOUZA, D.R.C. and QUEIROZ, S.C.N., 2021. Avaliação in vitro de atividade antimicrobiana de óleos essenciais contra Salmonella typhimurium e Staphylococcus aureus. *Revista Higiene Alimentar*, vol. 35, no. 293, p. e1060.

RAJASEKHARAN, S.K., RAMESH, S., SATISH, A.S. and LEE, J., 2017. Antibiofilm and anti-β-lactamase activities of burdock root extract and chlorogenic acid against Klebsiella pneumoniae. *Journal of Microbiology and Biotechnology*, vol. 27, no. 3, pp. 542-551. http://dx.doi.org/10.4014/jmb.1609.09043. PMid:27974734.

RAMALHO, M.A.S., SANTOS, B., RAMALHO, D.F., CUNHA, S.M.D., ANJOS, R.M., OLIVEIRA, H.M.B.F., SOUSA, A.P. and OLIVEIRA FILHO, A.A., 2020. Antiadherent activity of essencial oils of Eucalyptus globulus and Eucalyptus citriodora against Klebsiella pneumoniae strains. *Research*. *Social Development*, vol. 9, no. 7, pp. 1-15.

ROSATO, A., SBLANO, S., SALVAGNO, L., CAROCCI, A., CLODOVEO, M.L., CORBO, F. and FRACCHIOLLA, G., 2020. Anti-biofilm inhibitory synergistic effects of combinations of essential oils and antibiotics. *Antibiotics*, vol. 9, no. 10, p. 637. http://dx.doi.org/10.3390/antibiotics9100637. PMid:32987638.

SANTANA, M.T.P., GOMES, L.L., SANTOS, T.A., SOUSA, A.P., ALVES, M.A.S.G. and OLIVEIRA FILHO, A.A., 2020. Atividade antibacteriana do óleo essencial de Lavandula híbrida grosso associado à ampicilina contra cepas de Staphylococcus aureus. *Revista Brasileira de Educação e Saúde*, vol. 10, no. 4, pp. 96-100. http://dx.doi.org/10.18378/rebes.v10i4.7955.

SANTOS, D.F., 2007. *Características microbiológicas de Klebsiella pneumoniae isoladas no meio ambiente hospitalar de pacientes com infecção nosocomial*. Goiânia: Universidade Católica de Goiás. Dissertação de Mestrado em Ciências Ambientais e Saúde.

SARTORATTO, A., MACHADO, A.L.M., DELARMELINA, C., FIGUEIRA, G.M., DUARTE, M.C.T. and REHDER, V.L., 2004. Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Brazilian Journal of Microbiology*, vol. 35, no. 4, pp. 275-280. http://dx.doi.org/10.1590/S1517-83822004000300001.

SEIBERT, G., HORNER, R., MENEGHETTI, B.H., RIGHI, R.A., FORNO, N.L.F.D. and SALLA, A., 2014. Infecções hospitalares por enterobactérias produtoras de *Klebsiella pneumoniae* carbapenemase em um hospital escola. *Einstein (Sao Paulo, Brazil)*, vol. 12, pp. 282-286. http://dx.doi.org/10.1590/s1679-45082014ao3131. PMid:25295446.

SOUZA, E., CRUZ, J.H.A., FERREIRA, J.L.S., OLIVEIRA, H.M.B.F. and OLIVEIRA FILHO, A.A., 2021. Potencial antimicrobiano e antiaderente do óleo essencial de Lavandula híbrida grosso contra cepas de Klebsiella pneumoniae. *Archives of Health Investigation*, vol. 10, no. 6, pp. 906-912. http://dx.doi.org/10.21270/archi.v10i6.5008.

ULTEE, A., BENNIK, M.H.J. and MOEZELAAR, R., 2002. The phenolic hydroxyl group of carvacrol is essential for action against the food-borne pathogen Bacillus cereus. *Applied and Environmental Microbiology*, vol. 68, no. 4, pp. 1561-1568. http://dx.doi.org/10.1128/AEM.68.4.1561-1568.2002. PMid:11916669.

UZAIR, B., NIAZ, N., BANO, A., KHAN, B.A., ZAFAR, N., IQBAL, M., TAHIRA, R. and FASIM, F., 2017. Essential oils showing in vitro anti MRSA and synergistic activity with penicillin group of antibiotics. *Pakistan Journal of Pharmaceutical Sciences*, vol. 30, suppl. 5, pp. 1997-2002. PMid:29105634.

VAN DEN DOOL, H. and KRATZ, P.D., 1963. A generalization of retention index system including linear temperature programed gas-liquid partition chromatography. *Journal of Chromatography*.

A, vol. 11, pp. 463-471. http://dx.doi.org/10.1016/S0021-9673(01)80947-X.

WERT, L.A.D., RENSEN, S.S., SOONS, Z., POEZE, M., BOUVY, N.D. and PENDERS, J., 2020. The cutaneous microbiome in hospitalized patients with pressure ulcers. *Scientific Reports*, vol. 10, no. 1, p. 5963. http://dx.doi.org/10.1038/s41598-020-62918-8. PMid:32249804.

ZAPIÉN-CHAVARRÍA, K.A., PLASCENCIA-TERRAZAS, A., VENEGAS-ORTEGA, M.G., VARILLAS-TORRES, M., RIVERA-CHAVIRA, B.E., ADAME-GALLEGOS, J.R., GONZÁLEZ-RANGEL, M.O. and NEVÁREZ-MOORILLÓN, G.V., 2019. Susceptibility of multidrug-resistant and biofilm-forming uropathogens to Mexican oregano essential oil. *Antibiotics*, vol. 8, no. 4, p. 186. http://dx.doi.org/10.3390/antibiotics8040186. PMid:31618938.

ZENGIN, H. and BAYSAL, A.H., 2014. Antibacterial and antioxidant activity of essential oil terpenes against pathogenic and spoilage-forming bacteria and cell structure-activity relationships evaluated by SEM microscopy. *Molecules*, vol. 19, no. 11, pp. 17773-17798. http://dx.doi.org/10.3390/molecules191117773. PMid:25372394.