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Evaluation of the antibacterial, modulatory and anti-adherent properties of oregano *(Origanum vulgare)* essential oil against food pathogenic bacteria

Avaliação das propriedades antibacteriana, moduladora e antiaderente do óleo essencial de orégano *(Origanum vulgare)* contra bactérias patógenas de alimentos

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Highlights

Oregano essential oil (EO) demonstrates strong antibacterial activity.
The EO displays bactericidal potential against strains isolated from meat foods.
Oregano EO has a synergistic modulating effect when associated with antimicrobials.
Oregano EO shows anti-adherent activity against strains of *Pseudomonas aeruginosa.*

Abstract

There are over 250 types of foodborne diseases, the majority of which are infections caused by bacteria. *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus* are considered contaminants of meat products. The use of natural products as antimicrobials to combat these diseases can be an effective and economical approach. This study proposes to assess the antibacterial, modulatory, and anti-adherent activity of the essential oil of *Origanum vulgare* against strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus saprophyticus* isolated from meat products. The assay was conducted in duplicate. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined using the broth microdilution technique. MIC represents the

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lowest concentration of the product capable of inhibiting the growth of the bacterial strain, whereas MBC represents the lowest concentration capable of inhibiting total growth. The study of association of the product with antimicrobials was undertaken by disk diffusion using ampicillin, gentamicin, ceftazidime, and ciprofloxacin, resulting in synergistic, antagonistic, or indifferent effects. Anti-adherent activity was determined in the presence of sucrose, as the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube. Oregano oil exhibited strong inhibitory and bactericidal activity against *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus,* with MIC values ranging from 32 to 512 µg mL^{-1} and MBC values ranging from 128 to 512 µg mL^{-1}. *Origanum vulgare* oil showed varied interactions when associated with antimicrobials, with modulations for synergism (37.5%), indifference (50%), and antagonism (12.5%). Regarding anti-adherent activity, the test product effectively inhibited the adherence of *P. aeruginosa* bacterial strains in the presence of sucrose (1:8) but had no effect against *K. pneumoniae* or *S. saprophyticus.* Therefore, oregano oil proves to be an antibacterial and modulating agent against different bacteria isolated from meat products. Additionally, it displays anti-adherent properties against *P. aeruginosa,* making it a natural product that could serve as an interesting alternative in efforts to combat foodborne diseases.
Key words: Biology. Microbiology. One health. Phytotherapy.

Resumo

Existem mais de 250 tipos de doenças transmitidas por alimentos e a maioria são infecções causadas por bactérias, sendo *Klebsiella pneumoniae, Pseudomonas aeruginosa* e *Staphylococcus saprophyticus,* consideradas contaminantes de produtos cárneos. A utilização de produtos naturais como antimicrobianos para combater essas doenças, pode ser uma abordagem eficaz e econômica. O objetivo da presente pesquisa foi verificar a atividade antibacteriana, moduladora e antiaderente do óleo essencial de *Origanum vulgare* frente às cepas de *Klebsiella pneumoniae, Pseudomonas aeruginosa* e *Staphylococcus saprophyticus* isoladas de produtos cárneos. O ensaio foi realizado em duplicata. A Concentração Inibitória Mínima (CIM) e a Concentração Bactericida Mínima (CBM) foram determinadas através da técnica de microdiluição em caldo, sendo a CIM dada pela menor concentração do produto capaz de inibir o crescimento da cepa bacteriana, e a CBM pela menor concentração capaz de inibir o crescimento total. O estudo de associação do produto com os antimicrobianos foi realizado por difusão em disco utilizando ampicilina, gentamicina, ceftadizima e ciprofloxacino, tendo como resultado o efeito sinérgico, antagônico ou indiferente. A atividade antiaderente foi determinada na presença de sacarose, sendo determinada pela menor concentração do agente em contato com sacarose que impediu a aderência ao tubo de vidro. O óleo de orégano mostrou uma forte atividade inibitória e bactericida contra *Klebsiella pneumoniae, Pseudomonas aeruginosa* e *Staphylococcus saprophyticus,* com os valores de CIM variando entre 32 a 512 µg mL^{-1}, e de CBM entre 128 a 512 µg mL^{-1}. O óleo de *O. vulgare* apresentou interações variadas na associação com os antimicrobianos, com modulações para sinergismo (37,5%), indiferença (50%) e antagonismo (12,5%). Em relação a atividade antiaderente, o produto teste foi eficaz na inibição a aderência das cepas bacterianas de P. aeruginosa, na presença de sacarose (1:8), porém não houve efeito frente a *K. pneumoniae* e a *S. saprophyticus.* Portanto, o óleo de orégano apresenta-se como agente antibacteriano e modulador frente a diferentes bactérias isoladas de produtos cárneos, além de ser antiaderente frente a *P. aeruginosa,* sendo um produto natural que pode representar uma alternativa

interessante nos esforços para combater doenças transmitidas por alimentos.
Palavras-chave: Biologia. Fitoterapia. Microbiologia. Saúde única.

Introduction

Meat products, widely consumed globally, are recognized as one of the most important traditional food groups, providing essential nutritional compounds such as proteins, fats, essential amino acids, as well as selected minerals, and vitamins (Biesalski, 2005). The quality control of these products is crucial for preserving human health (Medvedevskikh et al., 2019).

Throughout the stages of food preparation, contamination can occur, leading to the development of foodborne diseases (Flores & Melo, 2015). Food- and waterborne diseases (FWBD) not only have negative effects on health and well-being but also result in adverse economic consequences for individuals, families, communities, businesses, and countries (World Health Organization [WHO], 2015).

There are over 250 types of FWBDs, predominantly infections caused by bacteria and their toxins (Brasil, 2018). Several bacteria species are pathogenic to humans (Meireles, 2017), including *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus,* found in meat products (Calbo et al., 2011; Á. F. S. Silva et al., 2022; Charmpi et al., 2020).

Klebsiella pneumoniae and *Pseudomonas aeruginosa* are Gram-negative species (Podschum & Ullmann, 1998; Fair & Tor, 2014), associated with clinical manifestations such as hospital-acquired respiratory and urinary tract infections (Oliveira et al., 2013; Breidenstein et al., 2011). *Staphylococcus saprophyticus,* coagulase-negative and novobiocin-resistant, is frequently isolated from urinary tract infections in young, sexually active women (Pead et al., 1980).

To combat bacterial diseases, the use of antimicrobial agents stands out as a primary therapeutic approach (Steiner, 2013). Although the discovery and development of antimicrobials have improved the control of infectious diseases, the triumph of this antibacterial therapy was short-lived (Kamaruzzaman et al., 2017). However, the overconsumption of antimicrobials, their use as growth promoters in livestock, and their largely uncontrolled release into the environment have led to the development of resistance (Tomley & Shirley, 2009), necessitating alternative strategies.

Combination therapy, incorporating essential oils and pharmacological methods, emerges as an effective and cost-efficient approach against antimicrobial resistance (Hashempour-Baltork et al., 2019). One such example of this method is combining essential oils with antimicrobials.

Recent studies demonstrate that oregano essential oil exhibits inhibitory effects against pathogenic bacteria isolated from meat products, including *Salmonella enterica* and *Listeria monocytogenes* (Vivian et al., 2020).

Origanum vulgare, commonly known as oregano, is a medicinal, aromatic, and spicy plant belonging to the family Lamiaceae, native to Europe and cultivated in southern and southeastern Brazil (Sakurai et al., 2016).

The biological activity of oregano oil is ascribed to its primary constituents, thymol and carvacrol, along with the presence of γ-terpinene, linalool, and ρ-cymene (Cunha et al., 2012).

Given the inhibitory effect of oregano spice on bacterial growth, this study explores the antibacterial, modulating, and anti-adherent potential of O. vulgare essential oil against strains of *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus* isolated from meat products.

Material and Methods

Test product

The *Origanum vulgare* (oregano) essential oil used originated from QUINARÍ® (Ponta Grossa – Paraná - latitude 25°13', longitude – 50°01'). It was dissolved in the presence of the dispersing agents Tween 80 (polysorbate 80) and DMSO (dimethyl sulfoxide), and diluted in distilled water (Allegrini et al., 1973).

Microorganisms

Strains of *Klebsiella pneumoniae* (Kp 42 and Kp 44), *Pseudomonas aeruginosa* (Pa 43 and Pa 44), and *Staphylococcus saprophyticus* (Sa 41 and 45) isolated from animal-derived food were employed. These strains, stored in the Microbiology Laboratory of the Academic Unit of Biological Sciences (UACB) at the Federal University of Campina Grande (UFCG) in Patos - PB, were maintained on Muller-Hinton Agar (MHA) at 4 ºC.

Inocula were obtained from overnight cultures in MHA at 37 ºC and diluted in sterile 0.9% saline solution to achieve a final concentration of approximately 1.5 × 10^8 colony-forming units per mL, adjusted by turbidity compared to a suspension of barium sulfate and sulfuric acid in the 0.5 tube of the McFarland scale (Bauer et al., 1966; Cleeland & Squires, 1991).

Laboratory tests were conducted in the Microbiology Laboratory of UACB at UFCG/Patos - PB.

Antimicrobials

The antimicrobials tested against bacterial cultures were ampicillin (10 µg mL^{-1}), gentamicin (10 µg mL^{-1}), ceftazidime (30 µg mL^{-1}), and ciprofloxacin (5 µg mL^{-1}), following the recommendations of the Clinical and Laboratory Standards Institute [CLSI] (2018).

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration was determined using the microdilution technique in a U-shaped bottom 96-well plate. In each well, 100 µL Mueller Hinton broth (doubly concentrated) and 100 µL of oregano essential oil at concentrations ranging from 1024 to 16 µg mL^{-1} were added. The MIC determination involved inoculating 10 µL of the microorganism in each well, approximately 1.5 × 108 colony-forming units per milliliter. The penultimate well, containing 200 µL of broth, served as the growth control, while the last well received only 200 µL of broth, serving as the negative control. The

assay was performed in duplicate, and the plates were incubated at 35 °C for 24 h.

After the proper incubation time, the first reading of the results was taken. Subsequently, 20 µL of sodium resazurin solution (SIGMA) in sterile distilled water at a concentration of 0.01% (w/v) were added, serving as a colorimetric oxide-reduction indicator for bacteria. The reading was performed visually by assessing the absence or presence of microorganism growth through the formation of a cluster of cells (button) and by observing the color change of the solution from blue to pink, indicating growth. A new incubation was carried out at 37 ºC. The MIC was determined as the lowest concentration of the essential oil inhibiting visible growth of the microorganism and the change in coloration of the solution from blue to pink, indicating growth (Palomino et al., 2002; Ostrosky et al., 2008; CLSI, 2012; Bona et al., 2014).

Determination of minimum bactericidal concentration (MBC)

Following the initial reading of results, inoculations (10 µL) of three dilutions from the MIC were made into Mueller-Hinton broth medium (100 µL per well) in a sterile microdilution plate for MBC determination. After incubation at 37 °C for 24 h, 20 µL of resazurin were added. The assays were further incubated at 37 ºC for another 24 h to confirm the concentration capable of inhibiting the total growth of bacterial species, verified by the absence of a change in the coloration of the indicator dye (Ncube et al., 2008; Guerra et al., 2012).

Study of association of the product with antimicrobials

To study the association of the product with antimicrobials, the solid medium disk diffusion technique using filter paper disks was employed (Bauer et al., 1966; Oliveira et al., 2006). A 20-µL aliquot of the MIC of the test product was transferred to the discs containing antimicrobials at their respective concentrations and then placed in smooth sterile Petri dishes (140 × 15) containing the MHA medium, which were previously inoculated with sterile swabs using an approximate volume of 1 mL of the bacterial suspensions. Subsequently, the plates were incubated at 37 °C for 24-48 h, followed by reading (Koneman et al., 2008; Ostrosky et al., 2008; Oliveira et al., 2006). The interfering effect of the combination of the product plus antimicrobials was evaluated according to the methodology described by Cleeland and Squires (1991). A halo of inhibition of microbial growth formed by the combined application of the essential oil (EO) and the antimicrobial (AB) was considered synergistic when its diameter was ≥ 2 mm compared to the halo of inhibition formed by the action of the AB alone. When the formation of a halo of inhibition resulting from the combined action of AB and EO had a smaller diameter than the one developed by the action of AB alone, it was considered an antagonistic effect. It was considered an indifferent effect when a halo of inhibition was observed as a consequence of the combined application of EO and AB with a diameter equal to that of the application of AB alone.

Determination of minimum inhibitory concentration of adherence (MICA)

The minimum inhibitory concentration of adherence (MICA) of the compound was determined in the presence of 5% sucrose, following the methodology of Albuquerque et al. (2010), using concentrations corresponding to the compound up to a 1:1024 dilution. For bacterial growth, the bacterial strain was cultured at 37 ºC in Mueller Hinton broth. Subsequently, 0.9 mL of the subculture was distributed in test tubes, and 0.1 mL of the solution corresponding to the dilutions of the compound was added. Incubation was carried out at 37 ºC for 24 h with tubes tilted at 30º. The reading was performed by visually observing the adherence of the bacteria to the walls of the tube after shaking it. The assay was conducted in duplicate. The same procedure was performed for the positive control, 0.12% chlorhexidine digluconate (Periogard®, Colgate-Palmolive Company, New York, USA). The MICA was considered the lowest concentration of the agent in contact with sucrose that prevented adherence to the glass tube.

Results and Discussion

The results of the antibacterial activity of *O. vulgare* essential oil, including MIC and MBC values, are presented in Table 1. The activity was assessed based on the presence of microorganism growth.

Table 1
Antibacterial activity of *Origanum vulgare* essential oil against *K. pneumoniae, P. aeruginosa* and *S. saprophyticus* isolated from meat products.

Bacterial strain	MIC	MBC	Negative Control	Positive Control
K. pneumoniae Kp 42	256 µg mL^{-1}	256 µg mL^{-1}	-	+
K. pneumoniae Kp 44	256 µg mL^{-1}	256 µg mL^{-1}	-	+
P. aeruginosa Pa 43	256 µg mL^{-1}	512 µg mL^{-1}	-	+
P. aeruginosa Pa 44	256 µg mL^{-1}	512 µg mL^{-1}	-	+
S. saprophyticus Sa 41	256 µg mL^{-1}	256 µg mL^{-1}	-	+
S. saprophyticus Sa 45	32 µg mL^{-1}	128 µg mL^{-1}	-	+

(-) there was no bacterial growth; (+) there was bacterial growth.

Upon analysis of Table 1, it was observed that oregano essential oil exhibited MIC values ranging from 32 to 256 µg mL^{-1} against the growth of different strains of *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus.* The oil demonstrated strong inhibitory activity, considering that the test substance is considered to have strong activity when the MIC falls within the range

of 50 to 500 µg mL^{-1}; moderate activity from 600 to 1500 µg mL^{-1}; and weak activity above 1500 µg mL^{-1} (Sartoratto et al., 2004).

The oil also displayed MBC values between 128 and 512 µg mL^{-1}, indicating bactericidal activity for most strains. According to Hafidh et al. (2011), a compound is considered bactericidal or bacteriostatic based on the ratio of MBC to MIC. When this ratio is between 1:1 and 2:1, the compound is considered bactericidal, and to be considered bacteriostatic, the ratio should be greater than 2:1.

The observed strong inhibitory activity and bactericidal/bacteriostatic effect of oregano essential oil against strains of *K. pneumoniae, P. aeruginosa,* and *S. saprophyticus* from meat products may be attributed to the composition of this substance. Silva et al. (2023) identified a total of nine constituents in *O. vulgare* oil from the Quinari brand, namely, alpha-pinene, camphene, myrcene, carene, cymene, gamma-terpinene, linalool, carvacrol, and caryophyllene, with carvacrol being the major compound at a high percentage (40.52%). Carvacrol is known to damage the lipid plasma membranes of bacteria, hindering cell division and causing cell dehydration (Araújo & Longo, 2016).

The MIC values were lower against the *S. saprophyticus* species, which may be linked to the greater sensitivity of Gram-positive bacteria compared to Gram-negative bacteria (Hyldgaard et al., 2012). According to Carvalho et al. (2019), this sensitivity is associated with the absence of an outer membrane in Gram-positive bacteria, allowing hydrophobic molecules to penetrate cells more easily, whereas Gram-negative bacteria possess an outer membrane that acts as a barrier against macromolecules and hydrophobic compounds.

Additional research supports the antibacterial activity of oregano oil against *Klebsiella pneumoniae.* Mohsen et al. (2022) reported effective results against *K. pneumoniae* (MDR) at concentrations of 62.5, 125, 250, and 500 µg mL^{-1} using clinical isolates and the agar well diffusion method. Another study tested four strains of *K. pneumoniae* isolated from clinical samples, using the microdilution technique, and found 75% inhibition at the concentration of 0.125% and 25% at the concentration of 0.25%, while the MBC occurred at the concentration of 0.5% (Costa et al., 2009).

The study by Leuthier et al. (2021) investigated the MIC using the microdilution method and the MBC employing the agar technique in Petri dishes. The obtained value for both concentrations against standard strains was 5000 µg mL^{-1}. Fournomiti et al. (2015) examined the impact of oregano oil on strains isolated from patients in a hospital in Greece and the standard strain (NCTC) of *K. pneumoniae.* The broth microdilution method revealed an average MIC value of 73.5 µg mL^{-1} for oregano oil.

Additional studies on oregano oil against *Pseudomonas aeruginosa* demonstrated effective antibacterial activity. In an investigation using *P. aeruginosa* strains isolated from combat victims using broth microdilution assay, MIC ranged from 0.32 to 0.64 mg mL^{-1} (Lu et al., 2018). Costa et al. (2009), using the microdilution technique, tested four clinical strains of *P. aeruginosa* and detected 75% inhibition at a 0.5% concentration and 25% at 0.25%, with a MIC

of 0.5%. In a study involving American Type Culture Collection (ATCC) strains, Leuthier et al. (2021) reported a MIC of 4166.67 µg mL^{-1} using the microdilution method and a MBC of 5000 µg mL^{-1} with the Petri dish agar technique.

Other bacterial species isolated from food were also tested against the essential oil of *O. vulgare,* thereby corroborating the data from the present study and affirming the substance's antibacterial effect. Pellegrini et al. (2018), employing the broth microdilution technique, investigated the antibacterial impact of oregano oil against strains of *Pseudomonas fluorescens* (isolated from dairy products), *Staphylococcus aureus* (isolated from dairy products), *Brochothrix thermosphacta* (isolated from poultry meat), *Salmonella Enteritidis* and *Typhimurium* (isolated from dairy products), *Enterococcus faecium* (isolated from fish), and *Listeria* monocytogenes (isolated from dairy products). As a result, they obtained MICs in a broad spectrum of activity, ranging from 1.25 to 10 µg mL^{-1}, with the best result against *P. fluorescens,* with a MIC of 1.25 µg mL^{-1}. In another study, Rossi et al. (2018) observed additional MIC values using *O. vulgare* oil against *P. fluorescenses* strains isolated from mozzarella cheese. Utilizing the microdilution technique, they obtained MIC and MBC values from 10 to 40 µg mL^{-1}.

A study conducted by Čabarkapa et al. (2019) delved into the antibacterial effect of oregano oil against strains of *Salmonella* Enteritidis isolated from poultry. The microdilution method in 96-well plates revealed MIC and MBC values of 0.156 and 0.3125 µg mL^{-1}, respectively. Another investigation assessed the antibacterial activity of oregano oil using both disk diffusion and broth microdilution techniques against 25 strains of *Salmonella* isolated from turkey, chicken, calf burgers, and wild boar liver (Listorti et al., 2020). Inhibition zones ranging from 10.3 to 23.3 mm in diameter were observed, along with MIC and MBC values ranging from 0.012 to 0.2 %v/v (Listorti et al., 2020).

Using the disk diffusion test, Yasir et al. (2024) assessed the antibacterial efficacy of *O. vulgare* oil against multidrug-resistant strains isolated from raw milk, including *Escherichia, Enterobacter* spp., *Citrobacter* spp., *Proteus* spp., *Klebsiella* spp., and *Staphylococcus.* They recorded inhibition halos ranging from 12 to 18 mm in diameter, with the most substantial zone of inhibition observed against *Staphylococcus* (18 mm) and the smallest against Klebsiella spp (12 mm).

Table 2 displays the interference of oregano essential oil on the action of antimicrobials in clinical use. When comparing the diameters of the halos of bacterial growth inhibition in tests with antimicrobials alone and in combination with essential oils, it is evident that essential oils can influence the antibacterial potency of antimicrobials in some interactions (Oliveira et al., 2006). This interference manifested as modulations for synergism, indifference, and antagonism, accounting for 37.5%, 50%, and 12.5%, respectively.

Ampicillin, gentamicin, and ceftazidime were the antimicrobials that exhibited the most significant interference from essential oils, demonstrating synergism in all three bacterial species under study, with at least one strain in each species. There was no interference of *O. vulgare* essential oil on ampicillin or ciprofloxacin in the majority of the strains under investigation.

In this study, the impact of the test product in combination with antimicrobials against *P. aeruginosa* aligns with the findings of Oliveira et al. (2006). They conducted an association study using the essential oils of *Conyza bonariensis, Lippia sidoides,* and *Eucalyptus citriodora,* each with the antimicrobials ampicillin, cephalothin, chloramphenicol, gentamicin, and tetracycline, against standard *P. aeruginosa,* resulting in indifference in the tested associations (Oliveira et al., 2006).

Table 2
Interference of *Origanum vulgare* essential oil in association with clinical use antimicrobials on *K. pneumoniae, P. aeruginosa* and *S. saprophyticus.* Average of duplicates

Antimicrobials		Microorganisms					
		K. pneumoniae Kp 42	*K. pneumoniae* Kp 44	*P. aeruginosa* Pa 43	*P. aeruginosa* Pa 44	*S. saprophytic* Sa 41	*S. saprophytic* Sa 45
Ampicillin	HI	0 mm	0 mm	0 mm	14 mm	28 mm	28 mm
	HIORI	0 mm *	10 mm ↑	0 mm *	16 mm ↑	0 mm ↓	30 mm ↑
Gentamicin	HI	20 mm	18 mm	18 mm	20 mm	30 mm	28 mm
	HIORI	20 mm *	20 mm ↑	20 mm ↑	20 mm *	30 mm *	30 mm ↑
Ceftazidime	HI	24 mm	24 mm	24 mm	28 mm	20 mm	10 mm
	HIORI	28 mm ↑	22 mm ↓	24 mm *	30 mm ↑	20 mm *	14 mm ↑
Ciprofloxacin	HI	32 mm	30 mm	30 mm	34 mm	34 mm	34 mm
	HIORI	30 mm ↓	30 mm *	30 mm *	36 mm *	34 mm *	34 mm *

HI: diameter of the growth inhibition zone in mm. HIORI: diameter of the growth inhibition halo determined by the association *Origanum vulgare.* HI: diameter of the growth inhibition zone determined by the antibiotic alone. Synergistic effect (↑); Antagonistic effect (↓); Indifferent effect (*).

The antibiotic modulation technique, involving the combination of a plant extract with a specific antibiotic to enhance its therapeutic potential, serves as a cost-effective and highly efficient alternative. It is simple to replicate, and due to the natural component added at low concentrations, it minimizes undesirable effects (Tintino et al., 2013).

As regards the results of MIC of *O. vulgare* against *K. pneumoniae, P. aeruginosa,* and *S. saprophyticus* adherence, it is evident that the oil effectively inhibited the adherence of *P. aeruginosa* bacterial strains in the presence of sucrose. Table 3 illustrates the minimum adherence inhibitory concentrations of this phytotherapeutic agent. The antibacterial agent (0.12% chlorhexidine digluconate) also showed significant inhibition in the adhesion effect against *S. saprophyticus,* whereas no inhibition was observed for *K. pneumoniae* or *P. aeruginosa.*

Table 3
Minimum adhesion inhibitory concentration of *Origanum vulgare* and chlorhexidine against the bacteria *K. pneumoniae, P. aeruginosa* and *S. saprophyticus*

Bacterial strain	*O. vulgare*	*Chlorhexidine*
Klebsiella pneumoniae Kp 42	-	-
Pseudomonas aeruginosa Pa 43	1:8	-
Staphylococcus saprophyticus Sa 45	-	1:8

(-) showed biofilm formation.

In the present study, oregano essential oil demonstrated efficacy in inhibiting the adhesion of *P. aeruginosa* bacterial strains, aligning with the findings of Lu et al. (2018). Their research achieved complete inactivation of the biofilm of this species, originating clinically, as well as *A. baumannii* and MRSA. The biofilm matrix's barrier effect can impede drug penetration significantly (Penesyan et al., 2015); nevertheless, these results suggest that oregano oil can overcome the obstacles posed by *P. aeruginosa, A. baumannii,* and MRSA biofilms, eliminating bacteria within the planktonic bacteria (Lu et al., 2018).

Using the microtiter plate method, Rossi et al. (2018) illustrated the promising role of oregano essential oil as a biofilm inhibitory agent against *Pseudomonas fluorescens* at a low temperature (10 °C). Čabarkapa et al. (2019) tested oregano oil against *Salmonella enteritidis* strains isolated from poultry and found that it inhibited biofilm formation in microtiter plates. Oregano oil also exhibited an antibiofilm effect on microtiter plates against strains of *Staphylococcus aureus* isolated from goat's milk (Ersanli et al., 2023) and, through the disk diffusion test, against strains of *S. aureus* and *Escherichia coli* isolated from raw milk "Minas" cheese (Campos et al., 2022). The efficacy of this oil against biofilm formation was previously reported by Oral et al. (2010) through the microtiter plate method, on *S. aureus, S. lugdunensis, S. haemolyticus, S. sciuri,* and *E. coli.*

Biofilms, defined as communities of microbial cells surrounded by a matrix of extracellular polymeric substances associated with biotic or abiotic surfaces (Sauer et al., 2007), are particularly persistent (Penesyan et al., 2015). They can serve as continuous sources of spoilage and pathogenic bacteria contaminating food (Oral et al., 2010). Given this, studies investigating the impact of natural plants on microbial biofilm formation through various mechanisms have gained relevance (Oral et al., 2010).

Conclusion

This study has established a theoretical framework that not only enhances the field of plant medicine but also lays the groundwork for forthcoming and imperative investigations.

Oregano oil exhibited robust inhibitory activity against *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus,* with MIC values ranging from 32 to 256 μg mL^{-1}. It demonstrated a bactericidal effect for the majority of the tested strains, except for *S. saprophyticus* strain Sa 45, which showed bacteriostatic effect.

Origanum vulgare oil emerged as a viable avenue in the search for compounds that potentiate the action of antimicrobial drugs, as evidenced by its synergistic effects when combined with ampicillin, gentamicin, and ceftazidime.

Additionally, it displayed an anti-adherent effect against *Pseudomonas aeruginosa,* with a ratio of 1:8.

Finally, oregano oil showcased its role as an antibacterial agent against strains of *Klebsiella pneumoniae, Pseudomonas aeruginosa,* and *Staphylococcus saprophyticus* isolated from meat products, making it a natural product that might constitute an interesting alternative in efforts to combat foodborne diseases.

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References

Albuquerque, A. C. L., Pereira, M. S. V., Pereira, J. V., Pereira, L. F., Silva, D. F., Macedo-Costa, M. R., & Higino, J. S. (2010). Efeito antiaderente do extracto de *Matricaria recutita* Linn. nos microrganismos do biofilme dentário. *Revista de Odontologia da UNESP, 39*(1), 21-25.

Allegrini, J., Buochberg, M. S. de, & Maillols, H. (1973). Emulsõesd'huiles essentielles fabricação e aplicações em microbioloige. *Travaux de la Société de Pharmacie de Montpellier, 33*(1),73-86.

Araújo, M. M., & Longo, P. L. (2016). Teste da ação antibacteriana in vitro de óleo essencial comercial de *Origanum vulgare* (orégano) diante das cepas de *Escherichia coli* e *Staphylococcus aureus. Arquivos do Instituto Biológico, 83,* e0702014, 1-7. doi: 10.1590/1808-1657000702014

Bauer, A. W., Kirby, W. M. M., Sherris, J. C., & Turck, M. (1996). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology, 45*(4), 493-496. doi: 10.1093/ajcp/45.4_ts.493

Biesalski, H. K. (2005). Meat as a component of a healthy diet-are there any risks or benefits if meat is avoided in the diet? *Meat Science, 70*(3), 509-524. doi: 10.1016/j.meatsci.2004.07.017

Bona, E. A. M., Pinto, F. G. S., Fruet, T. K., Jorge, T. C. M., & 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, 81*(3), 218-225. doi: 10.1590/1808-1657001192012

Brasil. Ministry of Health (2018). *Epidemiological data - FWBDs from 2000 to 2018.* http://portal.saude.gov. br/portal

Breidenstein, E. B. M., Fuente-Núñez, C., & Hancock, R. E. W. (2011). *Pseudomonas aeruginosa:* all roads lead to resistance. *Trends in Microbiology, 19*(8), 419-426. doi: 10.1016/j.tim.2011.04.005

Čabarkapa, I., Čolović, R., Đuragić, O., Popović, S., Kokić, B., Milanov, D., & Pezo, L. (2019). Anti-biofilm activities of essential oils rich in carvacrol and thymol against *Salmonella* Enteritidis. *Biofouling, 35*(3), 361-375. doi: 10.1080/08927014.2019.1610169

Calbo, E., Freixas, N., Xercavins, M., Riera, M., Nicolás, C., Monistrol, O., Solé Mdel, M., Sala, M. R., Vila, J., & Garau, J. (2011). Foodborne nosocomial outbreak of SHV1 and CTX-M-15-producing *Klebsiella pneumoniae:* epidemiology and control. *Clinical Infectious Diseases, 52*(6), 743-749. doi: 10.1093/cid/ ciq238

Campos, A. C. L. P., Nandi, R. D. S., Scandorieiro, S., Gonçalves, M. C., Reis, G. F., Dibo, M., Medeiros, L. P., Panagio, L. A., Fagan, E. P., Kobayashi, R. K. T., & Nakazato, G. (2022). Antimicrobial effect of *Origanum vulgare* (L.) essential oil as an alternative for conventional additives in the Minas cheese manufacture. *LWT - Food Science and Technology, 157*(113063), 1-7. doi: 10.1016/j.lwt.2021.113063

Carvalho, M. I. P., Albano, H. C. P., & Teixeira, P. C. M. (2019). Influence of oregano essential oil on the inhibition of selected pathogens in "Alheira" during storage. *Acta Scientiarum Polonorum Technologia Alimentaria, 18*(1), 13-23. doi: 10.17306/J.AFS.2019.0624

Charmpi, C., Van Reckem, E., Sameli, N., Van der Veken, D., De Vuyst, L., & Leroy, F. (2020). The use of less conventional meats or meat with high ph can lead to the growth of undesirable microorganisms during natural meat fermentation. *Foods, 9*(10), 1386. doi: 10.3390/foods9101386

Cleeland, R., & Squires, E. (1991). Evaluation of new antimicrobials *in vitro* and in experimental animal infections. *Antibiotics in Laboratory Medicine, 3*, 739-787.

Clinical and Laboratory Standards Institute (2012). Dilution antimicrobial susceptibility testing methods for aerobically growing bacteria. approved standard (9th ed.). *Clinical and Laboratory Standards Institute, CLSI.* (Document CLSI MO7- A9).

Clinical and Laboratory Standards Institute (2018). *Performance standards for antimicrobial susceptibility testing* (28nd ed.). Clinical and Laboratory Standards Institute, CLSI.

Costa, A. C., Santos, B. H. C., Santos, L. Fo., & Lima, E. O. (2009). Antibacterial activity of the essential oil of *Origanum vulgare* L. (Lamiaceae) against bacterial multiresistant strains isolated from nosocomial patients. *Brazilian Journal of Pharmacognosy, 19*(1B), 236-241. doi: 10.1590/S0102-695X2009000200 010

Cunha, A. P., Roque, O. R., & Nogueira, M. T. (2012). *Plantas aromáticas e óleos essenciais - composição e aplicações.* Fundação Calouste Gulbenkian.

Ersanli, C., Tzora, A., Skoufos, I., Fotou, K., Maloupa, E., Grigoriadou, K., Voidarou, C., & Zeugolis, D. I. (2023). The assessment of antimicrobial and anti-biofilm activity of essential oils against Staphylococcus aureus strains. *Antibiotics, 12*(2), 384-401. doi: 10.3390/antibiotics12020384

Fair, R. J., & Tor, Y. (2014). Antibiotics and bacterial resistance in the 21st century. *Perspectives in Medicinal Chemistry, 6,* 25-64. doi: 10.4137/pmc.s14459

Flores, A. M. P. C., & Melo, C. B. (2015). Principais bactérias causadoras de doenças de origem alimentar. *Revista Brasileira de Medicina Veterinária, 37*(1), 65-72. https://rbmv.org/BJVM/article/view/361

Fournomiti, M., Kimbaris, A., Mantzourani, I., Plessas, S., Theodoridou, I., Papaemmanouil, V., Kapsiotis, I., Panopoulou, M., Stavropoulou, E., Bezirtzoglou, E. E., & 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, 26,* 23289. doi: 10.3402/mehd.v26.23289

Guerra, F. Q. S., Mendes, J. M., Oliveira, W. A., Costa, J. G. M., Coutinho, H. D. M., & Lima, E. O. (2012). Chemical composition and antimicrobial activity of *Cinnamomum zeylanicum* Blume essential oil on multi-drug resistant *Acinetobacter* spp. strains. *Revista de Biologia e Farmácia, 8*(1), 62-70.

Hafidh, R. R., Abdulamir, A. S., Vern, L. S., Abu Bakar, F., Abas, F., Jahanshiri, F., & Sekawi, Z. (2011). Inhibition of growth of highly resistant bacterial and fungal pathogens by a natural product. *The Open Microbiology Journal, 5,* 96-106. doi: 10.2174/1874285801105010096

Hashempour-Baltork, F., Hosseini, H., Shojaee-Aliabadi, S., Torbati, M., Alizadeh, A. M., & Alizadeh, M. (2019). Drug resistance and the prevention strategies in food borne bacteria: an update review. *Advanced Pharmaceutical Bulletin, 9*(3), 335-347. doi: 10.15171/apb.2019.041

Hyldgaard, M., Mygind, T., & Meyer, R. L. (2012). Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components. Review article. *Frontiers in Microbiology, 25*(2), 1-24. doi: 10.3389/ fmicb.2012.00012

Kamaruzzaman, N. F., Kendall, S., & Good, L. (2017). Targeting the hard to reach: challenges and novel strategies in the treatment of intracellular bacterial infections. *British Journal of Pharmacology, 174*(14), 2225-2236. doi: 10.1111/bph.13664

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Win, W. C. J. (2008). *Diagnóstico microbiológico* (6a ed.). Médica e Científica Ltda.

Leuthier, L. L., Silva, E. C. A., Almeida, A., Jr., Nunes, J. M. F. F., Sampaio, F. C., & Farias, I. A. P. (2021). Efeito do óleo essencial de *Origanum vulgare* L. e do carvacrol no crescimento de bactérias patogênicas da orofaringe. *Research, Society and Development, 10*(1), e45210111754. doi: 10.33448/rsd-v10i1.11754

Listorti, V., Battistini, R., Ercolini, C., Tramuta, C., Razzuoli, E., Vencia, W., Decastelli, L., Gallina, S., Masotti, C., & Serracca, L. (2020). *In vitro* susceptibility of multidrug resistant strains of *Salmonella* to essential oils. *Natural Product Communications, 15*(1), 1446-1462. doi: 10.1177/1934578X19878904

Lu, M., Dai, T., Murray, C. K., & Wu, M. X. (2018). Bactericidal property of oregano oil against multidrug-resistant clinical isolates. *Frontiers in Microbiology, 9,* 2329. doi: 10.3389/fmicb.2018.02329

Medvedevskikh, M. Y., Sergeeva, A. S., Krasheninina, M. P., Ostrikova, N. L., Semenova, A. A., & Kuznetsova, O. A. (2019). About the development of reference materials of meat and meat product composition. *Journal of Physics: Conference Series, 1420*(1), 012030, 1-2. doi: 10.1088/1742-6596/1420/1/012030

Meireles, D. R. P. (2017). *Avaliação da atividade farmacológica e toxicológica do flavonoide isolado de Lonchocarpus araripensis (Leguminosae): estudos in silico e in vitro.* Tese de doutorado, Universidade Federal da Paraíba, João Pessoa, PB, Brasil.

Mohsen, L., Jaber, H., & Kamel, W. M. (2022). Antibacterial activity of the essential oil isolated from *Origanum vulgare* L. (Lamiaceae) against multi-drug resistant bacteria. *International Journal of Drug Delivery Technology, 12*(1), 81-84. doi: 10.25258/ijddt.12.1.15

Ncube, N., Afolayan, S. A. J., & Okoh, A. I. (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. *African Journal of Biotechnology, 7*(12), 1797-1806. doi: 10.5897/AJB07.613

Oliveira, M. M. M., Brugnetra, F. D., & Piccoli, H. R. (2013). Biofilmes em indústrias de laticínios: aspectos gerais e uso de óleos essenciais como nova alternativa de controle. *Revista do Instituto de Laticínios Cândido Tostes, 68*(390), 65-73. doi: 10.5935/2238-6416.20130010

Oliveira, R. A. G., Lima, E. O., Viera, W. L., Freire, K. R. L., Trajano, V. N., Lima, I. O., Souza, E. L., Toledo, M. S., & Silva, R. N., Fº. (2006). Estudo da interferência de óleos essenciais sobre a atividade de alguns antibióticos usados na clínica. *Revista Brasileira de Farmacognosia, 16*(1), 77-82. doi: 10.1590/S0102-695X2006000100014

Oral, N. B., Vatansever, L., Aydin, B. D., Güven, A., & Gülmez, M. (2010). Effect of oregano essential oil on biofilms formed by Staphylococci and *Escherichia coli. Kafkas Universitesi Veteriner Fakultesi Dergisi, 16*(Suppl-A), S23-S29. doi: 10.9775/kvfd.2009.1147

Ostrosky, E. A., Mizumoto, M. K., Lima, M. E. L., Kaneko, T. M., Nishikawa, S. O., & Freitas, B. R. (2008). Métodos para avaliação da atividade antimicrobiana de determinação de concentração mínima inibitória (CMI) de plantas medicinais. *Brazilian Journal of Pharmacognosy, 18*(2), 301-307. doi: 10.1590/S0102-695X2008000200026

Palomino, J. C., Martin, A., Camacho, M., Guerra, H., Swings, J., & Portales, F. (2002). Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistence *Mycobacterium tuberculosis. Antimicrobial Agents and Chemoterapy, 46*(8), 2720-2722. doi: 10.1128/aac.46.8.2720-2722.2002

Pead, L., Maskell, R., Morris, J. *S. saprophyticus* a urinary patogen: a six year prospective survey. *The Journal of Infectious Diseases, 142,* 239-246, 1980.

Pellegrini, M., Ricci, A., Serio, A., Chaves-López, C., Mazzarrino, G., D'amato, S., Lo Sterzo, C., & Paparella, A. (2018). Characterization of essential oils obtained from abruzzo autochthonous plants: antioxidant and antimicrobial activities assessment for food application. *Foods, 7*(2), 19-33. doi: 10.3390/foods7020019

Penesyan, A., Gillings, M., & Paulsen, I. T. (2015). Antibiotic discovery: combatting bacterial resistance in cells and in biofilm communities. *Molecules (Basel, Switzerland), 20*(4), 5286-5298. doi: 10.3390/molecules20045286

Podschum, R., & Ullmann, U. (1998). Klebsiella spp. as nosocomial phathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clinical Microbiology Reviews, 11*(4), 589-603. doi: 10.1128/cmr.11.4.589

Rossi, C., Chaves-Lopez, C., Serio, A., Anniballi, F., Valbonetti, L., & Paparella, A. (2018). Effect of *Origanum vulgare* essential oil on biofilm formation and motility capacity of *Pseudomonas fluorescens* strains isolated from discoloured Mozzarella cheese. *Journal of Applied Microbiology, 124*(5), 1220-1231. doi: 10.1111/jam.13707

Sakurai, F. N. K., Estrela, C. A., Tamayo, M. S., Casseb, M. O., & Nakasato, M. (2016). The characterization of functional properties of aromatic herbs used in a hospital specialized in cardiopneumology. Demetra: *Food, Nutrition & Health, 11*(4), 1097-1113. doi: 10.12957/demetra.2016.18170

Sartoratto, A., Machado, A. L. M., Delarmelina, C., Figueira, G. M., Duarte, M. C. T., & Rehder, V. L. G. (2004). Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Brazilian Journal of Microbiology, 35*(4), 275-280. doi: 10.1590/S1517-83822004000300001

Sauer, K., Rickard, A. H., & Davies, D. G. (2007). Biofilms and biocomplexity. *Microbe, 2*(7), 347-353. doi: 10.1128/microbe.2.347.1

Silva, Á. F. S., Cunha, F. R., Silva, L. G., Alves, D. G. S., Gomes, G. Y. D. V., Soares, J. H. de O., Pinheiro, I. O., & Jácome, A. T., Jr. (2022). Impact of different marketing conditions on the bacteriological quality of meat products. *Research, Society and Development, 11*(9), e37211931988. doi: 10.33448/rsd-v11i9.31988

Silva, S. L., Araújo, F. S. M., Silva, P. O. A., Silva, E. V. A., Bezerra, M. M. S. L., Diniz, A. F., Oliveira, D. M., Jesus, H. O., Nascimento, B. B., Jr., Medeiros, L. A. D. M., & Oliveira, A. A., Fº. (2023). Evaluation of the antimicrobial effect of the *Origanum vulgare* L essential oil on strains of Klebsiella pneumoniae. *Brazilian Journal of Biology, 83,* e269317. doi: 10.1590/1519-6984.269317

Steiner, T. (2013). Treating foodborne illness. *Infectious Disease Clinics of North America, 27*(3), 555-576. doi: 10.1016/j.idc.2013.05.006

Tintino, S. R., Cunha, F. A. B., Santos, K. K. A., Guedes, G. M. M., Souza, C. E. S., Matias, E. F. F., Morais-Braga, M. F. B., Andrade, J. C., Costa, J. G. M., Freitas, M. A., & Coutinho, H. D. M. (2013). Atividade moduladora de extratos etanólico e hexânico de raiz de *Costus* cf. *arabicus* sobre drogas antimicrobianas. *Revista Brasileira de Biociências, 11*(2), 157-162. http://www.ufrgs.br/seerbio/ojs/index.php/rbb/article/ view/2316

TomLey, F. M., & Shirley, M. W. (2009). Livestock infectious diseases and zoonoses. *Philosophical Transactions of the Royal Society B, 364*(1530), 2637-2642. doi: 10.1098/rstb.2009.0133

Vivian, P. G., Mello, G., Porto, R., Timm, C. D., Gandra, E. A., & Freitag, R. A. (2020). Antimicrobial activity of essential oils of *Origanum vulgare* (oregano) and *Ocimum basilicum* (basil) and its application in meat sausage. *Brazilian Journal of Development, 6*(8), 62143-62156. doi: 10.34117/bjdv6n8-587

World Health Organization (2015). *WHO estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015.* https://apps.who.int/iris/handle/10665/199350

Yasir, M., Nawaz, A., Ghazanfar, S., Okla, M. K., Chaudhary, A., Al, W. T., Ajmal, M. N., Abdelgawad, H., Ahmad, Z., Abbas, F., Wadood, A., Manzoor, Z., Akhtar, N., Din, M., Hameed, Y., & Imran, M. (2024). Anti-bacterial activity of essential oils against multidrug-resistant foodborne pathogens isolated from raw milk. *Brazilian Journal of Biology, 84,* e259449. doi: 10.1590/1519-6984.259449