Antimicrobial activity of oregano essential oil (*Lippia graveolens* K.) from Saucillo, Chihuahua against pathogens isolated from bovine mastitis

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ABSTRACT
Objective: To evaluate the antimicrobial activity of oregano (*Lippia graveolens* Kunth) essential oil sourced from Saucillo, Chihuahua, against bacterial pathogens isolated from clinical cases of bovine mastitis.
Design/Methodology/Approach: Milk samples were collected from cows exhibiting clinical signs of mastitis and subjected to bacterial isolation and identification using standard microbiological techniques. Antibiotic susceptibility profiles were determined, and the antimicrobial activity of the essential oil was evaluated through the disk diffusion method at four concentrations: 25%, 50%, 75%, and 100%.
Results: The most frequently isolated bacterial species were *Staphylococcus aureus* and *Klebsiella pneumoniae*. The oregano essential oil demonstrated significant inhibitory activity against both species, with inhibition zones increasing proportionally to concentration, reaching maximum values at 75% and 100%. Negative controls (distilled water and DMSO) showed no inhibitory effect, confirming the efficacy of the essential oil.
Limitations/Implications: Antibiotic susceptibility testing revealed that although most bacterial isolates were susceptible to conventional antibiotics, *S. aureus* exhibited resistance to penicillin and ampicillin, underscoring the need for natural alternatives.
Findings/Conclusions: These results confirm the potential of *Lippia graveolens* essential oil as a natural or complementary antimicrobial agent for the control of bovine mastitis. Its application may contribute to reducing antibiotic overuse and support the adoption of more sustainable practices in dairy production.

Keywords: *Lippia graveolens*, essential oil, bovine mastitis, bacterial resistance, natural antimicrobial, *in vitro*.

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INTRODUCTION

Milk and its derivatives constitute a vital source of nutrients and represent one of the primary products generated by the livestock sector. However, dairy producers continually face the challenge of diseases that affect bovine health and reduce productivity. Among these, bovine mastitis stands out due to its high prevalence and economic impact, which has driven the search for alternatives to the intensive use of antibiotics, favoring the

application of natural products such as oregano (*Lippia graveolens* Kunth) essential oil (OEO) due to its antimicrobial properties. Early disease detection is a key element for understanding the causes and progression of major pathologies in dairy cattle (Le Blanc, 2006; Petersson *et al.*, 2018; Fetrow *et al.*, 2020; Nguyen *et al.*, 2022). In this context, bovine mastitis defined as inflammation of the mammary gland caused by infection or trauma alters milk composition and significantly reduces milk yield. This condition is among the most common health issues in dairy farms, affecting animal welfare and the profitability of the dairy industry worldwide (Ali *et al.*, 2021). It is estimated that 15% to 20% of dairy cows are affected by this disease annually (Neculai *et al.*, 2021). Due to its multifactorial nature, mastitis incidence depends on both the animal's immune defense mechanisms and environmental factors, as well as the virulence of the pathogens involved (Krömker & Leimbach, 2017). Over 140 microorganisms have been associated with its etiology (Radzikowski *et al.*, 2020), with the most common being *Staphylococcus aureus*, *Streptococcus agalactiae*, *Streptococcus uberis*, *Escherichia coli*, and *Klebsiella pneumoniae* (Klaas, 2018; Ashraf, 2020; Morales *et al.*, 2023). Bovine mastitis is a leading cause of economic losses in the dairy industry due to reduced milk production, discarded milk contaminated with antibiotics, and treatment-related costs. The indiscriminate use of antimicrobial drugs has led to the emergence of resistant strains, complicating disease control and posing public health risks due to potential antibiotic residues in dairy products. Given this scenario, the search for safe, effective, and sustainable natural alternatives has become a priority. Plant-derived essential oils are noteworthy for their antimicrobial potential and their capacity to act synergistically with antibiotics, helping reduce therapeutic doses and mitigate the risk of bacterial resistance. In Mexico, *Lippia graveolens* Kunth (Mexican oregano) is a species of significant economic and biological value, widely distributed in arid and semi-arid regions, including the municipality of Saucillo, Chihuahua. Oregano exhibits strong antioxidant and antimicrobial properties against pathogenic microorganisms (Arcila-Lozano *et al.*, 2004), largely due to its main active compounds, thymol and carvacrol. These compounds disintegrate the outer membrane of Gram-negative bacteria, releasing lipopolysaccharides and increasing cytoplasmic membrane permeability to ATP, leading to pore formation and bacterial lysis (Abdul *et al.*, 2017). However, information on its efficacy against mastitis-causing bacteria remains limited.

Evaluating the antimicrobial activity of *Lippia graveolens* essential oil against pathogens isolated from bovine mastitis will generate scientific evidence supporting its potential use as an alternative or complementary agent to conventional treatments. Moreover, the findings may contribute to the development of more sustainable disease control strategies in dairy cattle, promoting food safety and improving the competitiveness of the livestock sector.

The essential oil was extracted by steam distillation using a VEVOR apparatus. The plant material was exposed to steam for three hours to release volatile compounds, which were subsequently condensed into the hydrosol. The oregano essential oil (OEO) was extracted using hexane in a separation funnel to isolate the organic phase containing the oil. The hexane was then removed using a rotary evaporator (model SM100-PRO, Science Med, Finland), and the oil obtained was stored in amber glass bottles at 4 °C until use.

Figure 1. Oregano sample from Saucillo, Chihuahua, used for essential oil extraction.

Bacterial strain isolation

Bacterial strains were isolated from milk samples collected from cows showing clinical signs of mastitis specifically, udder lesions (erythema and visible trauma) and serous-like milk secretions in the affected quarters. The samples, containing *Staphylococcus aureus* and *Klebsiella pneumoniae*, were obtained from the dairy herd of the Faculty of Agricultural and Forestry Sciences (FCAyF), Autonomous University of Chihuahua (UACH). Clinical suspicion was based on local signs of inflammation in the udder and observable changes in milk composition.

Sample collection was performed under aseptic conditions. After cleaning the teat area with 70% ethanol, milk was collected into sterile containers labeled with the cow ID, affected quarter, date, and time of sampling. All samples were transported under refrigeration and processed in the laboratory for microbiological analysis and bacterial identification. Microbiological analyses were conducted in the Physical Chemistry and Instrumental Analysis Laboratory of FCAyF. Blood agar, MacConkey agar, and Mannitol Salt agar were used for culturing. Samples were incubated for 24 hours at 37 °C, followed by Gram staining. The isolates were identified using the DADE BEHRING MicroScan autoSCAN-4 system (Siemens, USA). For *K. pneumoniae*, the NUC 86 panel (Beckman Coulter, USA) was used for Gram-negative bacteria, while for *S. aureus*, the Combo Type 44 panel (Beckman Coulter, USA) was used for Gram-positive bacteria. Additionally, a biochemical profile analysis was performed in triplicate (Tables 1 and 2), using Beckman Coulter LabPro software, version 4.43 (2021).

Antimicrobial sensitivity testing with oregano essential oil (oeo) - mcfarland standard

Following identification, isolated colonies of *Staphylococcus aureus* and *Klebsiella pneumoniae* were selected and suspended in sterile distilled water. The inoculum density was normalized at 620 nm using a GENESYS™ 20 spectrophotometer (Thermo Scientific, USA) and adjusted to match a 0.5 McFarland standard, corresponding to approximately 1.2×10^8 colony-forming units per milliliter (CFU/mL) (NCCLS, 2003). Four treatments (T1-T4) were prepared using oregano essential oil (OEO) at concentrations of 25%, 50%, 75%, and 100% (v/v), diluted in dimethyl sulfoxide (DMSO). These concentrations allowed

Table 1. Identification substrates for Gram-positive bacteria with Type 44 panel.

Test	Abbreviation	Test	Abbreviation
Crystal Violet	CV	Urea	URE
Micrococcus Screen	MS	Mannitol	MAN
Nitrate	NIT	Lactose	LAC
Novobiocin	NOV	Trehalose	TRE
PNPG	PGR	Mannose	MNS
Indoxyl Phosphatase	IDX	Sodium Chloride 6.5%	NaCl
Voges-Proskauer	VP	Sorbitol	SOR
Optochin	OPT	Arabinose	ARA
Phosphatase	PHO	Ribose	RBS

Table 2. Identification substrates for Gram negatives with NUC 86 panel.

Test	Abbreviation	Test	Abbreviation
Nitrate	NIT	Esculin	ESC
Glucose	GLU	Voges-Proskauer	VP
Sucrose	SUC	Citrate	CIT
Sorbitol	SOR	Malonate	MAL
Raffinose	RAF	ONPG	ONPG
Rhamnose	RHA	Tartrate	TAR
Arabinose	ARA	Acetamide	ACE
Indole	IND	O/F Glucose	OF/G
Adonitol	ADO	O/F Base	OF/B
Melibiose	MEL	Decarboxylase Base	DCB
Urease	URE	Arginine	ARG
Hydrogen Sulfide (H_2S)	H2S	Ornithine	ORN
Tryptophan Deaminase	TDA	Cetrimide	CET

for a gradual assessment of antimicrobial activity and the establishment of a potential dose-response relationship. DMSO was chosen for its ability to dissolve lipophilic compounds without interfering with bacterial growth at the tested concentrations. Two negative controls were included: sterile distilled water and 99% DMSO, to ensure that the observed antimicrobial effects could be attributed solely to the essential oil. All treatments were applied in triplicate using 6 mm diameter Whatman #3 filter paper discs impregnated with the respective test solutions. The inoculum consisted of 100 μL of a bacterial suspension standardized according to the McFarland tube, applied onto Mueller-Hinton agar plates. The inoculum was spread using a sterile swab with three passes across the agar surface, rotating the plate 60° between passes to ensure uniform distribution. Once the discs were placed on the agar, the plates were incubated upside down at 37 °C for 24 hours. After incubation, inhibition zone diameters were measured in millimeters using a caliper, recording two perpendicular axes. If no inhibition was observed, a value of 0 mm was recorded. Each condition was tested in triplicate. All four

concentrations of OEO were evaluated against both bacterial strains, with distilled water and DMSO serving as negative controls.

Statistical analysis

Statistical analysis of the mean inhibition zones was performed using SAS software, version 9.3. To determine significant differences between essential oil concentrations, an analysis of variance (ANOVA) was conducted, followed by Tukey's multiple comparison test at a significance level of $p<0.05$.

Minimum inhibitory concentration (MIC) determination

The MIC is defined as the lowest concentration of an antimicrobial agent that prevents visible bacterial growth under standardized conditions. In this study, MIC was determined only for antibiotics using commercial panels with the MicroScan autoSCAN-4 system. Plates were incubated for 24 hours at 36 °C, and MIC values were reported according to current Clinical and Laboratory Standards Institute (CLSI) guidelines. MIC was not determined for the essential oil; its antimicrobial activity was assessed solely through the disk diffusion method.

RESULTS AND DISCUSSION

Macroscopic and microscopic identification of *Staphylococcus aureus* and *Klebsiella pneumoniae*

Figure 2 shows the macroscopic and microscopic identification of bacterial strains isolated from milk samples. On blood agar, characteristic colonies of *S. aureus* were observed, and Gram staining confirmed their coccoid morphology in clusters and Gram-positive staining. In contrast, *K. pneumoniae* colonies were isolated on MacConkey agar, identified by their typical growth pattern on this selective medium. Gram staining revealed bacillary morphology and Gram-negative characteristics for this strain.

Table 4 presents the results of biochemical testing using the PC 44 panel for *S. aureus*. The results obtained with the PC 44 panel including catalase and coagulase production, nitrate reduction, and sugar fermentation (*e.g.*, mannitol and lactose) are well documented (Geary & Stevens, 1986; Chakraborty *et al.*, 2011). In addition, *S. aureus's* ability to reduce nitrates to nitrites is a known trait supported by previous research (Karmakar *et al.*, 2016). Its metabolic activity is further reflected in its capacity to ferment trehalose and ribose (Chakraborty *et al.*, 2011). These findings are consistent with earlier studies used to differentiate *S. aureus* from other species based on metabolic and enzymatic profiles.

Table 3. Treatments used in antimicrobial sensitivity testing with oregano essential oil (OEO).

Substance	Treatment	Concentration (%)	OEO (μL)	DMSO (μL)	Volume (μL)
OEO	1	25%	2.5	7.5	10
	2	50%	5.0	5.0	10
	3	75%	7.5	2.5	10
	4	100%	10.0	0.0	10

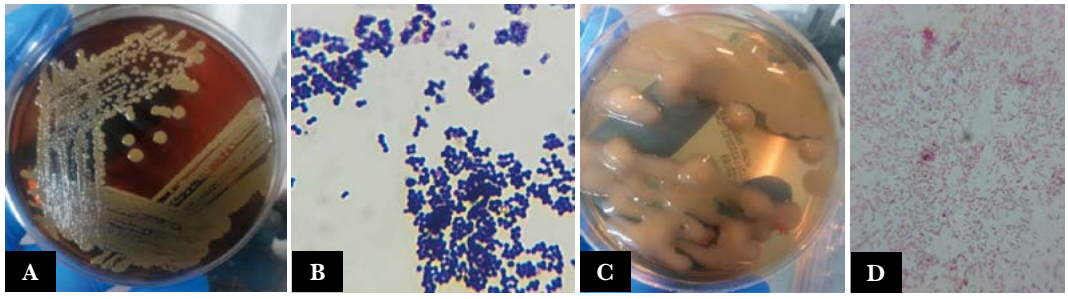

Figure 2. Macroscopic and microscopic identification of bacterial strains: (A) Staphylococcus aureus cultured on blood agar, (B) Gram-positive *S. aureus*, (C) *Klebsiella pneumoniae* cultured on MacConkey agar, (D) Gram-negative *K. pneumoniae*.

Table 4. Biochemical profile of *Staphylococcus aureus* isolated from bovine milk, obtained using the PC 44 panel. The results reflect the presence (+) or absence (−) of metabolic or enzymatic activity associated with each test.

Test	Interpretation	Test	Interpretation
Crystal Violet (CV)	−	Urease (URE)	+
Micrococcus Screen (MS)	+	Mannitol (MAN)	+
Nitrate (NIT)	+	Lactose (LAC)	+
Novobiocin (NOV)	−	Trehalose (TRE)	+
PNPG (PGR)	−	Mannose (MNS)	+
Indoxyl Phosphatase (IDX)	+	NaCl 6.5%	+
Voges-Proskauer (VP)	+	Sorbitol (SOR)	−
Optochin (OPT)	+	Arabinose (ARA)	−
Phosphatase (PHO)	+	Ribose (RBS)	+
Bile Esculin (BE)	−	Inulin (INU)	−
PYR	−	Raffinose (RAF)	−
Arginine (ARG)	+	Bacitracin (BAC)	+
PGT	+	PRV	−

Biochemical identification of *Klebsiella pneumoniae*

The biochemical analysis performed using the NUC 86 panel confirmed the identification of *Klebsiella pneumoniae*, with results consistent with the species' known metabolic and enzymatic characteristics. The bacterium tested positive for nitrate reduction, and fermentation of glucose, sucrose, and mannitol, as well as urease production, aligning with findings reported by Boye and Hansen (2004). Additionally, the strain showed the ability to metabolize citrate, malate, arabinose, indole, and acetamide reflecting the metabolic versatility of the species, particularly its capacity to utilize various carbon sources, a key feature of its pathogenicity (Moreno *et al.*, 2000). Conversely, the strain showed no production of hydrogen sulfide or phenylalanine deaminase activity and had limited capacity to ferment tartrate. These traits further support its identification and differentiation from other pathogens (Table 5). The biochemical profile of *K. pneumoniae* was established based on its metabolic and enzymatic responses.

Table 5. Biochemical test results for *Klebsiella pneumoniae* using the NUC 86 panel.

Test	Interpretation	Test	Interpretation
Nitrate (NIT)	+	Esculin (ESC)	+
Glucose (GLU)	+	Voges–Proskauer (VP)	–
Sucrose (SUC)	+	Citrate (CIT)	+
Sorbitol (SOR)	+	Malonate (MAL)	+
Raffinose (RAF)	+	ONPG	+
Rhamnose (RHA)	+	Tartrate (TAR)	–
Arabinose (ARA)	+	Acetamide (ACE)	–
Indole (IND)	+	O/F Glucose (OF/G)	+
Adonitol (ADO)	+	O/F Base (OF/B)	–
Melibiose (MEL)	+	Decarboxylase Base (DCB)	–
Urease (URE)	+	Arginine (ARG)	–
Hydrogen Sulfide (H_2S)	–	Ornithine (ORN)	–
Inositol (INO)	–	Tryptophan Deaminase (TDA)	–
Lysine (LYS)	+	Cetrimide (CET)	–

Minimum inhibitory concentration (MIC) as a standard for antimicrobial susceptibility MIC values serve as the gold standard for determining the susceptibility of a bacterial strain to a specific antimicrobial agent. The MIC is defined as the lowest concentration of the antimicrobial compound required to inhibit visible bacterial growth. The antibiogram results for *Staphylococcus aureus* (Table 6) reveal a high level of susceptibility to various antibiotics, with most agents classified as sensitive (S). For example, antibiotics such as amoxicillin/clavulanic acid, ampicillin/sulbactam, ceftriaxone, ciprofloxacin, clindamycin, and levofloxacin showed MIC values ≤ 4 μg/mL, demonstrating strong effectiveness against *S. aureus*, in line with findings reported in the literature (Batista *et al.*, 2019; El Behiry *et al.*, 2012). However, penicillin and ampicillin displayed MIC values >8 μg/mL, indicating resistance. This is consistent with the known production of β-lactamases in certain *S. aureus* strains, as described by Boyce and Medeiros (1987). Additionally, antibiotics such as rifampicin, linezolid, and vancomycin exhibited MIC values ≤ 1 μg/mL, confirming their high efficacy against *S. aureus*. This observation is supported by studies like that of Usha and Shwetha (2018), which emphasize the effectiveness of these agents in treating staphylococcal infections. The resistance to penicillin and ampicillin is likely due to the expression of β-lactamase genes, a well-documented phenomenon in *S. aureus* strains (Boyce & Medeiros, 1987).

The antibiogram of *Klebsiella pneumoniae* using the NUC 86 panel showed high sensitivity to amikacin, cephalosporins, carbapenems, and fluoroquinolones (Table 7). These results indicate that the tested strain had not acquired resistance mechanisms such as carbapenemase or β-lactamase production.

The intermediate susceptibility to aztreonam may be attributed to the presence of β-lactamases that compromise its clinical efficacy (Rodríguez-Baño *et al.*, 2018). The observed sensitivity to carbapenems likely reflects the absence of carbapenemase-

Table 6. Antibiogram results for *Staphylococcus aureus* strain.

Antimicrobial Agent	MIC (μg/mL)	Interpretation
Amoxicillin/Clavulanic Acid	≤4/2	S
Ampicillin/Sulbactam	≤8/4	S
Ampicillin	>8	BLAC
Ceftriaxone	≤1	S
Ciprofloxacin	≤1	S
Clindamycin	≤0.5	S
Daptomycin	≤0.5	S
Erythromycin	≤0.5	S
Gentamicin	≤4	S
Levofloxacin	≤1	S
Linezolid	2	S
Moxifloxacin	≤0.25	S
Nitrofurantoin	≤32	S
Oxacillin	≤0.25	S
Penicillin	>8	BLAC
Rifampicin	≤1	S
Cefoxitin Screening	≤4	NEG
Synercid	≤0.5	S
Tetracycline	≤4	S
Trimethoprim/Sulfamethoxazole	≤0.5/9.5	S
Vancomycin	2	S

(S)=Susceptible; (R)=Resistant; (BLAC)=β-lactamase positive.

producing mechanisms, as highlighted by Logan and Weinstein (2017), who emphasized the effectiveness of these agents under such conditions. Moreover, the response to fluoroquinolones aligns with previous findings for non-multidrug-resistant strains (Pitout *et al*., 2015). These results reveal a broad sensitivity profile, offering multiple therapeutic options against *K. pneumoniae* strains isolated from bovine mastitis cases.

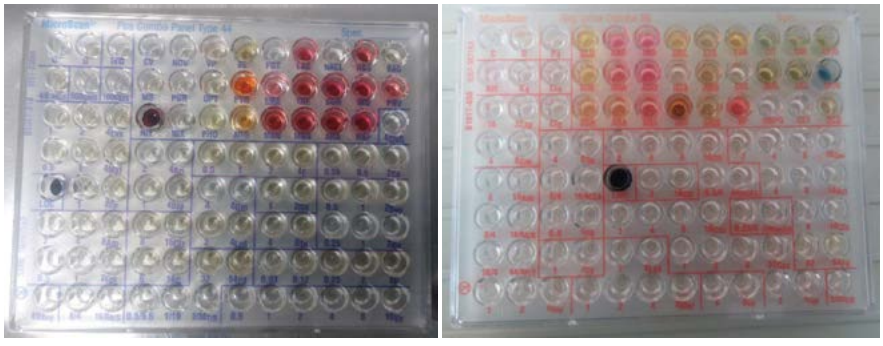

Figure 3. Biochemical and metabolic tests with antibiograms for (A) PC 44 panel for *S. aureus*, (B) NUC 86 panel for *K. pneumonia*.

Table 7. Antibiogram results for *Klebsiella pneumoniae* using the NUC 86 panel.

Antimicrobial Agent	MIC (μg/mL)	Interpretation
Amikacin	≤16	S
Ampicillin/Sulbactam	≤8/4	S
Aztreonam	16	I
Cefazolin	≤4	S
Cefepime	≤2	S
Cefotaxime	≤2	S
Cefotaxime/Clavulanic Acid	≤0.5	S
Cefotrixin	≤8	S
Ceftazidime	≤1	S
Ceftazidime/Clavulanic Acid	≤0.25	S
Ceftazidime/Avibactam	≤8	S
Ceftriaxone	≤1	S
Ciprofloxacin	≤1	S
Ertapenem	≤0.5	S
Gentamicin	≤4	S
Imipenem	≤1	S
Levofloxacin	≤1	S
Meropenem	≤1	S
Nitrofurantoin	≤32	S
Piperacillin/Tazobactam	≤16	S
Tetracycline	≤4	S
Tigecycline	≤4	S
Tobramycin	≤4	S
Trimethoprim/Sulfamethoxazole	≤2/38	S

(S) susceptible, (I) intermediate, (R) resistant.

Sensitivity testing with oregano essential oil (OEO)

The results demonstrate significant antimicrobial activity of OEO against both *Staphylococcus aureus* and *Klebsiella pneumoniae*. The antimicrobial effect was concentration-dependent, with larger inhibition zones observed at higher concentrations ranging from 21.8 mm to 35.4 mm for *S. aureus* and from 19.4 mm to 34.1 mm for *K. pneumoniae*. These findings are consistent with the work of Burt (2004). Negative controls (distilled water and DMSO) produced no inhibition zones, confirming that the antimicrobial activity observed was exclusively due to the OEO. Table 8 summarizes the treatments applied, bacterial strains tested, and mean inhibition zone diameters. When each species was analyzed separately, the 75% and 100% OEO concentrations produced significantly larger inhibition zones compared to the 25% and 50% concentrations for both *S. aureus* (groups A-D) and *K. pneumoniae* (groups a-e). As expected, the negative controls (water, DMSO) showed no inhibition (0.0 mm). These results align with Ríos *et al.* (2005), who reported strong

Table 8. Treatments, strains and means of the data obtained.

OEO Concentration (%)	*S. aureus* (mm)	*K. pneumoniae* (mm)
25	21.8±0.4 D	19.4±0.9 e
50	24.8±0.5 C	23.9±0.6 cd
75	31.3±0.8 B	29.1±0.6 b
100	35.4±0.7 A	34.1±0.7 a
Distilled Water	0.0±0.0 E	0.0±0.0 f

Note: Uppercase letters compare treatments within *S. aureus*; lowercase letters compare treatments within *K. pneumoniae* (ANOVA+Tukey, α=0.05).

activity of oregano essential oil against both Gram-positive and Gram-negative bacteria. Furthermore, even the lowest concentration tested (25%) demonstrated considerable antimicrobial activity, suggesting that OEO may be a viable option for bacterial infection control at moderate concentrations, as supported by Naveed *et al.* (2013). However, while antimicrobial activity was evident against both strains, the variation in inhibition zone size may reflect differences in bacterial cell wall structure or resistance mechanisms between Gram-positive and Gram-negative species, as previously noted by Chouhan *et al.* (2017).

When each species was analyzed separately, the 75% and 100% OEO concentrations produced significantly larger inhibition zones compared to the 25-50% concentrations for both *S. aureus* (A-D) and *K. pneumoniae* (a-e). Negative controls (water and DMSO) showed no inhibition (0.0 mm). Specifically, the 100% concentration yielded the largest inhibition zones: 35.4 mm for *S. aureus* and 34.1 mm for *K. pneumoniae*. These findings are consistent with those of Ríos *et al.* (2005), who reported strong antimicrobial activity of oregano essential oil against both Gram-positive and Gram-negative bacteria. Notably, even the lowest tested concentration (25%) produced considerable inhibition, suggesting that oregano essential oil may be a viable option for treating bacterial infections at moderate concentrations, as proposed by Naveed *et al.* (2013). However, the observed differences in inhibition zone sizes between the two species may reflect structural differences in the bacterial cell wall or the presence of resistance mechanisms, a phenomenon reported in other Gram-positive and Gram-negative strains (Chouhan *et al.*, 2017). As shown in Table 8, increasing the OEO concentration from 25% to 100% resulted in a progressive increase in inhibition zones from 21.8 to 35.4 mm for *S. aureus*, and from 19.4 to 34.1 mm for *K. pneumoniae*. The absence of inhibition with distilled water and DMSO confirms that the antimicrobial activity was attributable solely to the essential oil. This trend is consistent with recent studies showing that oregano essential oils, rich in thymol and carvacrol, display enhanced antimicrobial activity against both Gram-positive and Gram-negative bacteria in a concentration-dependent manner (Tejada-Muñoz *et al.*, 2024). The antimicrobial activity of thymol and carvacrol is primarily linked to their disruptive action on bacterial membranes. These compounds integrate into the lipid bilayer, increase membrane permeability, cause depolarization, and trigger ion leakage, thereby compromising cellular homeostasis (Khwaza & Aderibigbe, 2025). Their hydrophobic aromatic ring allows easy insertion into the bacterial membrane, while the −OH group facilitates proton

exchange. This dual mechanism disrupts lipid packing, increases membrane permeability, and destabilizes proton and voltage gradients critical for cell viability (Mączka *et al.*, 2023). Although Gram-negative bacteria are generally less susceptible due to their lipopolysaccharide (LPS) outer membrane, OEO at 75-100% still produced inhibition zones in *K. pneumoniae* comparable to those in *S. aureus*. Furthermore, recent studies have shown that oregano essential oil can synergize with antibiotics to reduce the viability of *Klebsiella*, effectively lowering the required therapeutic doses (Tao *et al.*, 2025).

Figure 5 shows sensitivity tests in Petri dishes with Mueller-Hinton agar showing the inhibition halo, (A) *S. aureus* sample, (B) *K. pneumoniae* sample, (C) Negative controls of distilled water and DMSO.

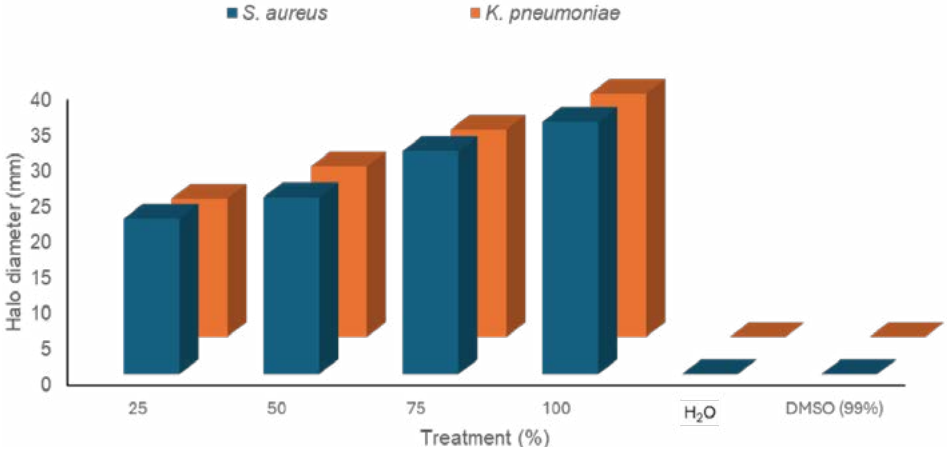

Figure 4. Antimicrobial sensitivity testing with oregano essential oil (OEO) against both bacterial strains.

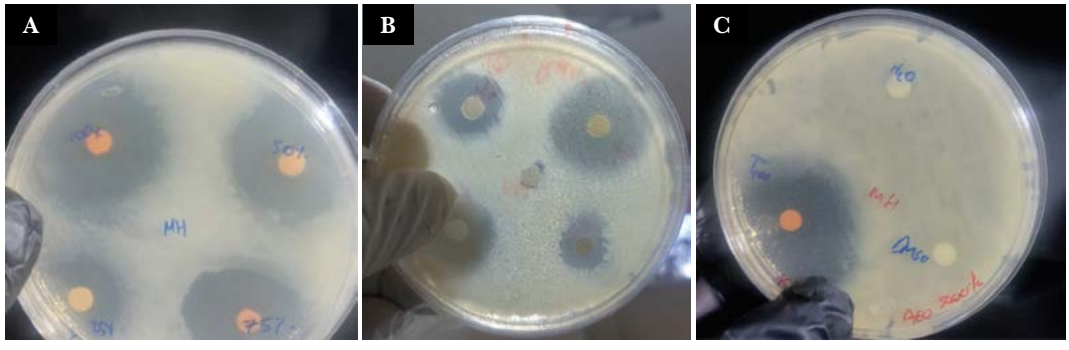

Figure 5. Sensitivity tests in Petri dishes with Mueller-Hinton agar showing the inhibition halo, (A) *S. aureus* sample, (B) *K. pneumoniae* sample, (C) Negative controls of distilled water and DMSO.

CONCLUSIONS

This study demonstrated that oregano essential oil (*Lippia graveolens* Kunth), sourced from Saucillo, Chihuahua, Mexico, exhibits notable antimicrobial activity against *Staphylococcus aureus* and *Klebsiella pneumoniae*, two pathogens commonly associated with bovine mastitis. The results revealed a directly proportional relationship between

oil concentration and inhibition zone size, with the highest values recorded at 75% and 100% concentrations. The essential oil showed comparable efficacy against both Gram-positive and Gram-negative bacteria, suggesting the presence of active compounds primarily thymol and carvacrol capable of disrupting bacterial membrane integrity, destabilizing ionic gradients, and ultimately inhibiting growth. The lack of inhibition in the negative controls (distilled water and DMSO) confirms that the observed antimicrobial activity is solely attributable to the essential oil. Furthermore, antibiotic susceptibility profiles indicated that the isolated strains remain sensitive to most conventional antimicrobials. However, the resistance to penicillin and ampicillin observed in *S. aureus* highlights the importance of exploring natural alternatives to help reduce the use of synthetic antibiotics and the selective pressure that drives bacterial resistance. These findings support the potential of *Lippia graveolens* essential oil as an alternative or complementary antimicrobial agent for the control of bovine mastitis. Its use could represent a sustainable and safe strategy, benefiting both animal health and the safety of dairy products. Nonetheless, further studies are recommended to assess its efficacy under *in vivo* conditions, determine optimal concentrations, evaluate stability, and examine any effects on milk quality. These steps are crucial for advancing toward the practical application of OEO in the prevention and treatment of bovine mammary infections, contributing to the development of more sustainable and competitive production systems.

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