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COMPARATIVE ANTIMICROBIAL EFFICACY OF LAVENDER AND MINT ESSENTIAL OILS: A PROMISING ALTERNATIVE FOR VETERINARY APPLICATIONS

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ABSTRACT

With the increasing prevalence of antimicrobial resistance (AMR) in veterinary pathogens, there is a growing need to explore alternative therapeutic options. This study presents a direct comparative analysis of the antimicrobial efficacy of mint and lavender essential oils (EOs) against key veterinary pathogens, including *Escherichia coli*, *Pasteurella multocida*, *Proteus mirabilis*, *Klebsiella pneumoniae*, *Corynebacterium pseudotuberculosis*, *Streptococcus agalactiae*, and methicillin-resistant *Staphylococcus aureus* (MRSA). Antimicrobial potency was investigated through the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), along with conducting time-kill assays and antibiotic interaction studies using disc diffusion and broth dilution methods. While both essential oils have potential as alternative antimicrobial agents, lavender EO stands out, especially against drug-resistant bacteria like MRSA. Lavender EO was more bactericidal, killing most isolates within four hours, whereas mint EO needed a full 24 hours for its full efficacy. MICs ranged between 12 and 40 µL/mL, with slightly lower MICs with lavender against *K. pneumoniae*. Noticeably, Lavender EO demonstrated a strong synergistic effect with antibiotics, particularly enhancing the efficacy of penicillin and tetracycline against MRSA. In contrast, mint EO exhibited only limited synergy and, at times, an antagonistic interaction. These findings highlight the superior antimicrobial potential of lavender EO over mint EO, underscoring its therapeutic value in veterinary medicine.

KEYWORDS: Essential oils, Veterinary pathogens, Synergistic interactions and Time –Kill assays

1. INTRODUCTION

In the past few years, in response to the rising problems posed by drug-resistant pathogens, there has been an increased effort towards finding antimicrobial candidates from naturally derived agents (Salam *et al*., 2023; Anwer *et al*., 2024). Among the natural alternatives, the essential oils have been enjoying a mercurial rise in popularity, primarily due to their sizable bioactive properties, coupled with their broad-spectrum activity against Gram-positive/negative bacteria and fungi (Issa, 2024). These essential oils are extracted from different plant parts, including leaves, flowers, or stems, consisting of complex mixtures of volatile aromatic compounds, endowed with their different fragrances, and diverse biological effects (Mohamed and Alotaibi, 2023). Antimicrobial activity thus provides these essential oils as possible candidates for replacing synthetic agents.

Mint oil from the species Mentha and lavender oil from the plant Lavandula angustifolia are the most famous with impressive activity though not exclusive (Hudz *et al.*, 2023; Posgay *et al*., 2022). Both the oils manifest substantial inhibitory properties against various bacterial and fungal pathogens, attributed to their rich bioactive constituents (Sriti *et al*., 2024). Mint essential oil is characterized by the great prevalence of menthol, menthone, and other monoterpenes imparting antibacterial and antiphlogistic functions (Semerdjieva *et al.*, 2024). All of those act to disrupt microbial cell membranes, inhibi crucial enzymes, and interfere with some cellular mechanisms, finally leading to microbial cell death (Pedroso *et al*., 2024).

Lavender essential oil, on the other hand, is known too for its antimicrobial properties, primarily from its active ingredients, linalool and linalyl acetate (Halat *et al*., 2022). These compounds possess broad-spectrum antimicrobial activity toward bacteria, fungi, and even certain viruses: the major mode of action consists of membrane disruption, enzyme inhibition and interference within the genetic material of the microbes, eventually leading to cell destruction (Imran *et al*., 2022).

Despite the growing interest in the antimicrobial effects of essential oils, comparative studies measuring the efficacy of various oils against clinically relevant veterinary pathogens are few. In this research, we attempt to address this knowledge gap by describing the antibiotic susceptibility profiles of these pathogens and testing for the antimicrobial properties of mint and lavender essential oils. Efficacies of these oils were determined employing minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and time-kill assays. Moreover, a comparative assessment between these two oils was performed.

2. MATERIALS AND METHODS

Ethical Approval

The Ethical Committee of the College of Veterinary Medicine at the University of Duhok in Iraq granted approval for the study to be conducted (Permit number: CVM2024/0110UoD).

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Study Period and Location

The research was carried out at the College of Veterinary Medicine, University of Duhok, Iraq, from September 2024 to February 2025.

Plant Materials and Essential Oil Extraction

Samples of mint (*Mentha spicata*) and lavender (*Lavandula angustifolia*) were sourced from farms in Duhok Province, Iraq, and a taxonomist from the College of Agricultural Engineering Sciences, University of Duhok, verified their botanical identification. To ensure quality, the samples were carefully cleaned and air-dried indoors. Essential oils were then extracted using a Clevenger apparatus, achieving a purity level exceeding 99%.

Antibiotic Discs

This study evaluated the antimicrobial susceptibility of bacterial isolates from animals using the disc diffusion method. A total of thirteen antibiotic discs were tested, including Penicillin (P), Ciprofloxacin (CIP), Ceftriaxone (CRO), Tetracycline (TE), Gentamicin (GN), Streptomycin (S), Chloramphenicol (C), Imipenem (IPM), Amoxicillin/Clavulanic Acid (AMC/AUG), Ceftiofur (CFT), Sulfamethoxazole/Trimethoprim (SMX-TMP), Azithromycin (AZM), and Tylosin (TY).

Bacterial Isolates

The bacterial isolates from veterinary clinical cases were isolated and identified using molecular techniques at the College of Veterinary Medicine, University of Duhok, Iraq. *Escherichia coli* (*E.coli*), *Pasteurella multocida* (*P. multocida*), *Proteus mirabilis* (*P. mirabilis*) and *Klebsiella pneumoniae* (*K. pneumoniae*) were isolated from pneumonic cases in sheep and goats slaughtered in abattoirs across Duhok Province (Ahmed and Abdullah, 2022). Two methicillin-resistant *S. aureus* (MRSA) strains, PQ881807 (MRSA-c) from a cat and PQ881808 (MRSA-d) from a dog, both associated with pneumonic cases, were isolated by Rasol and Abdulrahman, (2023). *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*) was recovered from sheep diagnosed with caseous lymphadenitis (Khanamir *et al*., 2023), and *Streptococcus agalactiae* (*S. agalactiae*) was isolated from cattle with mastitis (Amal *et al*., unpublished data).

Determination of the Antibiotic and EOs Sensitivity Profile

The antimicrobial sensitivity of microorganisms to both conventional antimicrobial agents and EOs was evaluated using the Kirby-Bauer method, with a slight modification (Hami and Ibrahim, 2023; F. A. Issa, 2024). Instead of antibiotic discs, 15 µL of pure EO was directly applied to culture plates. Bacterial cultures were inoculated on Mueller-Hinton agar (MHA), while *C. pseudotuberculosis* were grown on blood agar, with both incubated at 37°C for 24–48 hours. Observations were recorded and analyzed.

The results were interpreted in accordance with the guidelines specified for animal isolates by CLSI (Clinical and Institute, 2022). Isolates were categorized as either susceptible or resistant, with those exhibiting intermediate sensitivity to a particular antibiotic classified as resistant**.**

Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of Eos

The study employed serial dilution and viable colony counts, along with spectrophotometric methods (Martini *et al*., 2024), to quantify the colony-forming units (CFU) of bacteria. Bacterial cultures were grown in brain-heart infusion broth and incubated in a shaker incubator. Challenge doses of 5×10^6 CFU/mL were calculated using a calibration curve correlating $\log_{10}$ counts with optical density. Broth dilution testing (Issa, 2024) was used to evaluate the effectiveness of various essential oil (EO) concentrations against bacterial isolates.

For MIC determination, 1 mL of a 5×10^6 CFU/mL bacterial suspension was transferred to 1.5 mL microtubes, followed by the addition of EOs starting at concentration of 3 µL/ ml (V/V). The microtubes were vortexed and incubated at 37°C for 24–48 hours. The MIC was defined as the lowest EO concentration that prevented visible microbial growth. For MBC determination, 20 µL of suspensions from MIC tubes and subsequent dilutions were subculture onto MHA except for *C. pseudotuberculosis*, which was plated on blood agar. MBC concentrations were determined as the lowest EO concentrations that resulted in no microbial growth on the agar plates after 24–48 hours of incubation at 37°C (Asad *et al*., 2025).

Evaluation of the Bactericidal Activity of Essential Oil Using a Time-Kill Assay

The bactericidal activity of the tested EO was further assessed using a time-kill assay following the determination of its MBC. Bacterial suspensions at 5×10^6 CFU/mL were prepared, aliquoted into Eppendorf tubes, and treated with the EO at its MBC concentration. The mixtures were incubated at 37°C in a shaker incubator set to 150 rpm to ensure proper aeration and mixing.

At three-time intervals: 2 hours (short interval), 4 hours (medium interval), and 24 hours (long interval), aliquots were collected and at each interval, 20 µL of the suspension of the bacterial growth was plated onto MHA and *C. pseudotuberculosis* samples on blood agar. Plates were incubated at 37°C for 24–48 hours. Controls consisting of suspensions without EO were also included to monitor natural microbial growth over time.

Evaluation of the Antagonistic or Synergistic Effect of Antibiotics and EOs of Mint and Lavender on Bacterial Isolates:

The synergistic or antagonistic interactions between antibiotics (only those with inhibition zones of 10–15 mm) and the essential oils (EOs) of mint and lavender on bacterial isolates were evaluated by assessing their combined effectiveness. The EOs were used at a concentration of 6 µL (0.5 × MIC). Antibiotic discs were placed on cultivated bacterial cultures grown on Mueller-Hinton agar (MHA) and C. pseudotuberculosis samples on blood agar. Each disc was then saturated with 6 µL of essential oil to determine their combined antimicrobial effects. An increase of more than 2 mm in the inhibition zone diameter compared to individual agents was defined as synergism (Khleifat *et al*., 2019), while antagonism was characterized by a reduction of more than 2 mm (Sy *et al*., 2016).

Statistical Analysis

The zones of inhibition for the essential oils (EO) were compared with antibiotics that demonstrated activity against the tested veterinary bacterial strains. One-way ANOVA (GraphPad Prism 8.0.1) was used to assess significant differences ($p < 0.05$) among the antibiotics and the EOs. Additionally, the Chi-square test was employed to determine any differences in the bacterial cidal activities of the EOs at different time points. Data represent the mean ± SE of three independent experiments.

3. RESULTS

Antibiotics and Eos Sensitivity Profile

The antimicrobial susceptibility profiles of the tested bacteria are shown in (Table 1). The results revealed distinct susceptibility and resistance patterns among the isolates, highlighting both conventional and alternative antimicrobial agents (Figure 1).

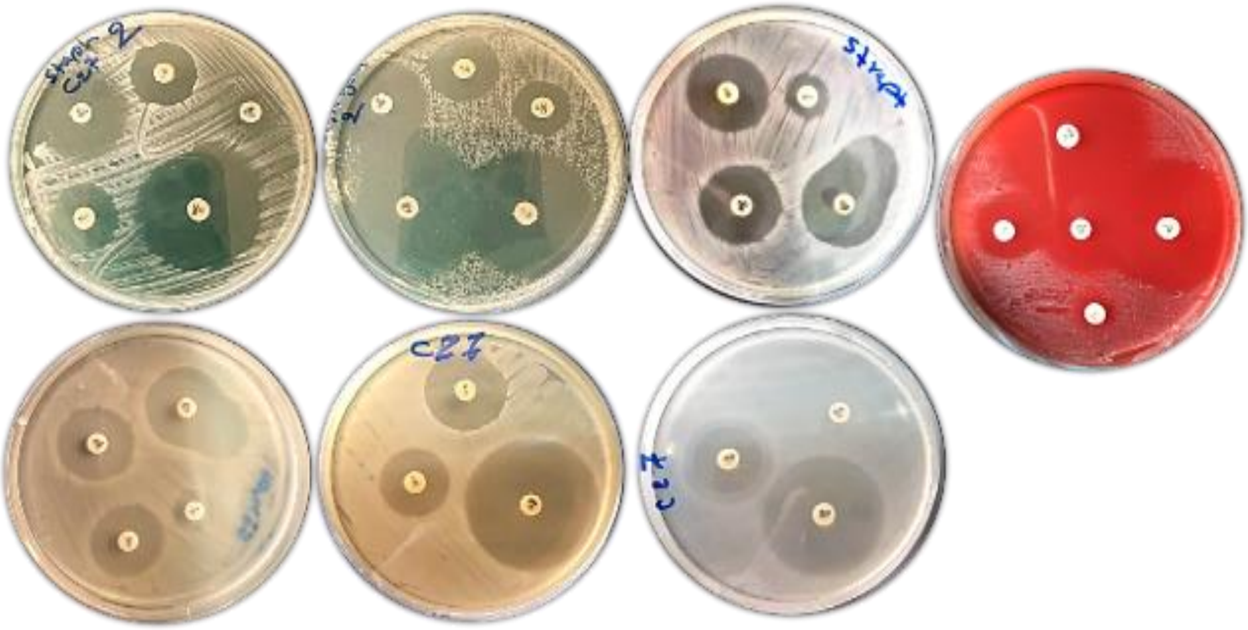

Figure 1: Sensitivity and resistance patterns of some of the tested bacteria using the disc diffusion method.

Penicillin resistance was observed in *E. coli*, *P. multocida*, *P. mirabilis*, *K. pneumoniae*, and *S. agalactiae*, with inhibition zones ranging from 0 mm to 13.0 ± 1.5 mm. In contrast, *C. pseudotuberculosis* remained susceptible, exhibiting an inhibition zone of 26.0 ± 3.5 mm. Ciprofloxacin demonstrated broad-spectrum efficacy, with all isolates except *P. mirabilis* showing susceptibility (inhibition zones: 21.0 ± 0.6 to 33.7 ± 3.5 mm). Ceftriaxone was effective against most Gram-negative isolates; however, resistance was noted in *C. pseudotuberculosis*, *S. agalactiae*, and MRSA strains.

Tetracycline and gentamicin exhibited limited efficacy, with universal resistance observed across all isolates. Streptomycin produced mixed results: *P. multocida*, *K. pneumoniae*, and *S. agalactiae* were susceptible, while other isolates, including MRSA strains, were resistant. Chloramphenicol was effective against most isolates except *P. multocida*, which displayed resistance. Imipenem demonstrated excellent activity, with complete susceptibility observed across all tested bacteria.

The efficacy of amoxicillin-clavulanic acid (AMC/AUG) varied: *E. coli*, *K. pneumoniae*, *C. pseudotuberculosis*, and *S. agalactiae* were susceptible, whereas *P. multocida*, *P. mirabilis*, and MRSA strains were resistant. Ceftiofur was effective against *P. multocida*, *P. mirabilis*, *K. pneumoniae*, and *S. agalactiae* but ineffective against *E. coli*, *C. pseudotuberculosis*, and MRSA-C1. Sulfamethoxazole-trimethoprim (SMX-TMP) yielded mixed outcomes, with susceptibility observed in *P. multocida*, *K. pneumoniae*, and MRSA strains, but resistance in *E. coli*, *P. mirabilis*, and *S. agalactiae*.

Azithromycin was effective against *P. multocida*, *P. mirabilis*, and *K. pneumoniae*, but resistance was noted in *E. coli*, *S. agalactiae*, and MRSA strains. Tylosin showed limited efficacy, with only *S. agalactiae* and MRSA-C27 displaying partial susceptibility.

Of particular interest, lavender and mint extracts exhibited significant antimicrobial activity, with all isolates demonstrating susceptibility. Inhibition zones ranged from 21.0 ± 1.5 mm (MRSA-C1) to 28.7 ± 0.7 mm (*E. coli*) for lavender and from 20.3 ± 1.2 mm (MRSA-C1) to 29.0 ± 0.6 mm (*C. pseudotuberculosis*) for mint. These findings highlight the potential of plant-based extracts as promising alternative antimicrobial agents, warranting further investigation into their therapeutic applications.

Table 1: Antimicrobial resistance and susceptibility patterns of veterinary bacterial isolates across different strains.

Used antibiotics	*E. coli*	*P. multocida*	*P. mirbilis*	*K. pneumonia*	*C. pseudotuberculosis*	*S. Agalactiae*	*MRSA- d*	*S aureus MRS-c*
Penicillin	R 0	R 6.3 ± 0.3	R 4.0 ± 0.6	S 13.0 ± 1.5	S 26.0 ± 3.5	R 10.3 ± 0.3	R 0	R 9.0 ± 1.0
Ciprofloxacin	S 28.3 ± 1.7	S 28.3 ± 1.7	R 10.3 ± 1.9	S 33.7 ± 3.5	S 30.3 ± 1.5	S 22.0 ± 0.6	S 26.0 ± 0.6	S 21.0 ± 0.6
Ceftriaxone	S 26.7 ± 2.4	S 27.7 ± 2.3	S 28.3 ± 1.7	S 27.7 ± 0.3	R 0	R 12.7 ± 0.3	R 10.3 ± 0.3	R 10.3 ± 0.3
Tetracycline	R 5.3 ± 0.3	R 0	R 0	R 5.0 ± 5.0	R 6 ± 0.6	R 0	R 13.3 ± 0.9	R 6.7 ± 1.3
Gentamicin	R 13.0 ± 0.6	R 12.0 ± 1.2	R 13.7 ± 0.3	R 14.7 ± 1.5	R 10 ± 1.2	R 9.0 ± 0.6	R 10.0 ± 2.9	R 10.7 ± 1.9

Streptomycin	R $^{L M}$ 15.5 ± 0.5	S 16.3 ± 2.6	R 14.0 ± 1.2	S 17.0 ± 1.5	R 8.5 ± 0.5	S ^{M} 20.3 ± 1.5	R 15.0 ± 0.6	R 10.0 ± 1.0
Chloramphenicol	S 27.5 ± 2.5	R 14.0 ± 2.0	S 24.3 ± 1.3	S 30.0 ± 1.7	S 30.3 ± 0.9	S 22.7 ± 0.3	S $^{L M}$ 21.0 ± 0.6	S 21.0 ± 0.6
Imipenem	S 28.3 ± 1.7	S 29.0 ± 1.0	S 30.7 ± 0.7	S 28.0 ± 1.2	S 31.7 ± 0.9	S 28.7 ± 0.9	S $^{L M}$ 20.3 ± 0.3	S 25.0 ± 0.6
AMC or AUG	S 23 ± 2.5	R 16.7 ± 0.7	R 17.3 ± 1.3	S 24.3 ± 1.2	S 25 ± 2.9	S 21.0 ± 0.6	R 15.3 ± 0.3	R 13.0 ± 0.6
Ceftiofur	R 6 ± 0.6	S 26.0 ± 3.1	S 25.7 ± 2.2	S 23.3 ± 4.4	R 10.3 ± 2.3	S ^{M} 27.3 ± 1.5	S 23.0 ± 1.2	R 0
SMX-TMP	R 0	S 27.0 ± 1.5	R 0	S 33.0 ± 3.8	S 18.0 ± 6.0	R 0	S ^{L} 22.3 ± 1.5	S 25.7 ± 1.3
AZM	R 1.3 ± 0.7	S 23.3 ± 2.0	S $^{L M}$ 19.3 ± 1.8	S 23.0 ± 2.0	S 18.3 ± 6.1	R 0	R 10.0 ± 2.9	R 0
Tylosin	R 0	R 0	R 0	R 0	R 0	R 7.3 ± 3.7	S 17.3 ± 3.9	R 15.3 ± 4.8
lavender	S 28.7 ± 0.7	S 28.3 ± 0.3	S 26.7 ± 1.3	S 28.0 ± 0.6	S 31.0 ± 0.6	S 23.3 ± 2.4	S 27.0 ± 0.6	S 21.0 ± 1.5
mint	S 27.0 ± 1.0	S 28.3 ± 0.3	S 27.7 ± 0.3	S 27.3 ± 0.7	S 29.0 ± 0.6	S 27.3 ± 0.7	S 26.3 ± 0.9	S 20.3 ± 1.2

The superscripts "L=lavender EO" and "M= Mint EO" indicate significant differences in the inhibitory zones (in mm) between the essential oils (EOs) of Mint and Thymus, as well as antibiotics. Asterisk notations (*, **, ***) correspond to the following levels of significance: $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively.

Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of EOs

The MIC was determined as the lowest EO concentration that prevented visible microbial growth (Figure 2). Mint and lavender EOs demonstrated similar antimicrobial activity against the tested animal-derived bacterial isolates. MICs ranged from 12 to 40 µL/mL, while MBCs ranged from 15 to 50 µL/mL. *P. mirabilis* and *Streptococcus agalactiae* exhibited the highest MIC and MBC values (40 and 50 µL/mL, respectively) for both EOs. *Klebsiella pneumoniae* showed a slightly lower MIC with lavender EO (12 µL/mL) compared to mint EO (15 µL/mL) (Table 2).

Table 2: Minimum inhibitory concentration and minimum bactericidal concentration values of mint and lavender essential oils against bacterial isolates of animal origin.

Bacterial Isolates	**Mint EO**		**Lavender EO**	
	MIC	**MBC**	**MIC**	**MBC**
E. coli	12	15	12	15
P. multocida	12	15	12	15
P. mirbilis	40	50	40	50
K. pneumonia	15	18	12	15
C. pseudotuberculosis	15	18	15	18
S. agalactiae	40	50	40	50
MRSA-d	12	15	12	15
MRSA-c	12	15	12	15

Figure 2: Broth macro-dilution to determine MIC of EO against *E. coli* (5×10^5 CFU/ml). Complete inhibition occurred at 12 µl EO /5×10^6 CFU/ml.

Time-Kill Kinetics of EOs Against the Tested Bacteria

The results of Table 3 illustrate the time required to kill bacterial isolates at the MBC using mint and lavender EOs. The time to bacterial death was recorded at 2 hours, 4 hours, and 24 hours post-treatment. Growth (G) indicates that the essential oil was unable to kill or eliminate the bacteria at the specified time, while no growth (NG) indicates successful elimination.

Mint EO demonstrated limited bactericidal activity at 2 hours post-treatment, as growth (G) was observed in all isolates, including *E. coli*, *K. pneumoniae*, *P. multocida*, *P. mirabilis*, *C. pseudotuberculosis*, *S. agalactiae*, and all MRSA isolates. By 4 hours, mint EO successfully eliminated *C. pseudotuberculosis* and *K. pneumoniae* (NG) but failed to eliminate the remaining isolates. At 24 hours, mint EO was effective in eliminating all isolates, indicating improved bactericidal activity over time. In contrast, lavender EO showed poor performance at 2 hours, failing to eliminate most isolates except *K. pneumoniae*. However, by 4 hours, lavender EO successfully eliminated all isolates except *E. coli*, *P. multocida*, and *P. mirabilis* that were successfully eliminated at 24 hours.

Overall, Mint EO demonstrated limited bactericidal activity at the earlier time points, with improved effectiveness observed at 24 hours. Lavender EO, on the other hand, consistently showed superior performance across most isolates, particularly at the 4-hour time point. However, the difference was not statistically significant, although elimination was observed for the majority of isolates. Both essential oils exhibited varying levels of efficacy, with Lavender EO performing more effectively at intermediate time points compared to Mint EO.

Table 3: Time required killing bacterial isolates at the minimum bactericidal concentration (MBC) for various bacterial isolates, using mint and lavender *EOs.* Time to bacterial death was recorded at 2 hours, 4 hours, and 24 hours post-treatment.

Bacterial Isolates	**2h post treatment**		**4h post treatment**		**24h post treatment**	
	Mint EO	Lavender Eo	Mint EO	Lavender Eo	Mint EO	Lavender Eo
E. coli	G	G	G	G	NG	NG
K. pneumonia	G	NG	NG	-	-	-
P. multocida	G	G	G	G	NG	NG
P. mirbilis	G	G	G	G	NG	NG
C. pseudotuberculosis	G	G	NG	NG	-	-
S. agalactiae	G	G	G	G	NG	NG
Staph aureus MRSA-d	G	G	G	NG	NG	-
Staph aureus MRSA-c	G	G	G	NG	NG	-

G= growth and NG= no growth.

Impact of the Interactions Between Antibiotics and EOs of Mint and Lavender on Bacterial Isolates:

For *K. pneumoniae*, both lavender and mint essential oils (EOs) exhibited antagonistic effects when combined with penicillin and gentamicin, resulting in smaller inhibition zones than those produced by the EOs alone. In contrast, for *S. agalactiae*, lavender EO demonstrated a synergistic effect with penicillin, yielding a zone of inhibition of 28.5 ± 1.5 mm. Mint EO, however, showed an indifferent effect, with no significant difference observed between the EO alone and its combination with penicillin (24.5 ± 0.5 mm). When combined with gentamicin, both EOs displayed antagonism, each producing inhibition zones of 11 ± 0 mm.

For MRSA-d, lavender EO showed synergy with tetracycline (30 ± 0 mm) and gentamicin (35 ± 1 mm), while mint EO was indifferent with tetracycline (26 ± 1 mm) and antagonistic with gentamicin (22.5 ± 0.5 mm). Both EOs were synergistic with AMC or AUM, producing zones of 32.5 ± 2.5 mm (lavender) and 30 ± 1 mm (mint).

For *E. coli*, both lavender and mint EO combinations with gentamicin were antagonistic, with zones of 20 ± 2 mm and 11 ± 1.41 mm, respectively. Similarly, for *P. multocida*, gentamicin combinations with both EOs were antagonistic, producing zones of 25.5 ± 0.5 mm (lavender) and 16 ± 1 mm (mint). Chloramphenicol and AMC or AUG combinations with both EOs were also antagonistic. For *C. pseudotuberculosis*, gentamicin combinations with both EOs were antagonistic, with zones of 20 ± 0 mm. In *P. mirabilis*, streptomycin and AMC or AUG combinations with both EOs were antagonistic, producing zones of 20.5 ± 0.5 mm (lavender) and 21 ± 1 mm (mint) for streptomycin, and 21.5 ± 1.5 mm (lavender) and 17.5 ± 0.5 mm (mint) for AMC or AUG.

Table 4: Antimicrobial activity of lavender EO and mint EO, alone and in combination with antibiotics, against bacterial isolates from animals. The inhibition zone diameters (mm) are presented as mean ± standard error.

Bacterial Isolates	Used antibiotics	Alone	lavender EO			Mint EO		
			Alone	Combination	*Results	Alone	Combination	results
		mm	mm	mm		mm	mm	
K.pneumonia	Penicillin	13.0 ± 1.5	28.0 ± 0.6	20.75 ± 0.8	A	27.3 ± 0.7	6.5 ±1.5	A
	Gentamicin	14.7 ± 1.5		10 ± 0	A		10 ± 0	A
S. agalactiae	Penicillin	10.3 ± 0.3	23.3 ± 2.4	28.5 ± 1.5	S	27.3 ± 0.7	24.5 ± 0.5	A
	Gentamicin	9.0 ± 0.6		11 ± 0	A		11 ± 0	A
MRSA-d	Tetracycline	13.3 ± 0.9	27.0 ± 0.6	30 ± 0	S	26.3 ± 0.9	26 ± 1	I
	Gentamicin	10.0 ± 2.9		35 ± 1	S		22.5 ± 0.5	A
	Streptomycin	15.0 ± 0.6		23 ± 1	A		23.5 ± 0.5	A
	AMC or AUM	15.3 ± 0.3		32.5 ± 2.5	S		30 ± 1	S
E. coli	Gentamicin	13.0 ± 0.6	28.7 ± 0.7	20 ± 2	A	27.0 ± 1.0	11 ± 1.41	A
P. multocida	Gentamicin	12.0 ± 1.2	28.3 ± 0.3	25.5 ± 0.5	A	28.3 ± 0.3	16 ± 1	A
	Chloramphenicol	14.0 ± 2.0		20 ± 1	A		11 ± 1	A
	AMC or AUG	16.7 ± 0.7		15.5 ± 0.5	A		18.5 ± 0.5	A
C.pseudotuberculosis	Gentamicin	10 ± 1.2	31.0 ± 0.6	20 ± 0	A	29.0 ± 0.6	20 ± 0	A
P. mirbilis	Streptomycin	14.0 ± 1.2	26.7 ± 1.3	20.5 ± 0.5	A	27.7 ± 0.3	21 ± 1	A
	AMC or AUG	17.3 ± 1.3		21.5 ±1.5	A		17.5 ± 0.5	A

* Results are classified as antagonistic (A) when the combination produced a smaller inhibition zone than either agent alone, synergistic (S) when the combination produced a larger inhibition zone, and indifferent (I) when there was no significant difference.

4. DISCUSSIONS

This study provided a detailed evaluation of the effectiveness of the antibiotics and mint/lavender oils against bacteria that infect animals, including both Gram-positive and Gram-negative types. The antibiotic susceptibility results underscore the growing challenge of antimicrobial resistance, with widespread resistance observed across multiple classes of antibiotics. Gram-negative bacteria, such as *E. coli, P. multocida, P. mirabilis, and K. pneumoniae*, showed resistance to penicillin, which is not surprising, as previous studies documented these bacteria's produce enzymes β-lactamases that break down penicillin (Trinchera *et al*., 2025). Imipenem and ciprofloxacin were effective against a wide range of these bacteria. This confirms their well-known role as go-to antibiotics for treating infections that resist multiple drugs (Eslami *et al*., 2025; Shariati *et al*., 2022). However, we found that all the bacteria tested were resistant to tetracycline and gentamicin. This is a significant problem, considering these antibiotics are frequently used in both animal and human medicine (Gasparrini *et al*., 2020; Zhang *et al*., 2023).

The lavender and mint essential oils observed strong antimicrobial activity, and all the tested bacteria were sensitive to them. These findings are in line with recently published studies that show plant-based essential oils can be powerful antimicrobials, likely due to their complex mix of natural chemicals like phenols, terpenes, and aldehydes (Di Matteo *et al*., 2024). Subtle differences were noticed concerning the oils' effectiveness against specific bacteria. Mint oil, which is high in menthol and menthone, was slightly better at killing *C. pseudotuberculosis*. On the other hand, lavender oil, with its linalool and linalyl acetate, was more effective against *E. coli*. These differences might be because the active compounds in each oil work in slightly different ways, such as by damaging bacterial membranes, blocking essential enzymes, or disrupting their communication systems (Guillín *et al*., 2021; Yap *et al*., 2021).

The antimicrobial effects of mint and lavender essential oils (EOs) were quite similar. However, the highest concentrations of EOs to stop and eliminate bacteria were observed in *P. mirabilis* and *S. agalactiae*, suggesting that these bacteria may have intrinsic resistance mechanisms, potentially involving efflux pumps or biofilm formation (Hajiagha and Kafil, 2023; Wasfi *et al*., 2020). Lavender EO demonstrated greater effectiveness against *K. pneumoniae,* requiring a lower concentration to inhibit its growth compared to mint EO. This is likely due to the active compounds of lavender EO, which can penetrate the bacterial cell wall more easily. These results support recent research showing that lavender EO has strong antimicrobial effects, particularly against Gram-negative bacteria (Kajjari *et al*., 2022).

Distinct differences in how mint and lavender essential oils (EOs) eliminated bacteria were observed in the time–kill assays. Lavender EO worked faster, where most bacterial isolates were eliminated within just four hours. In contrast, mint EO required a full 24 hours for complete bacterial eradication. The faster bacterial elimination could be due to the faster diffusion and interaction of lavender's active compounds with bacterial membranes (Batiha *et al*., 2023). The ability of lavender EO to perform well in shorter time frames suggests it could be particularly useful for treating acute infections, where quickly clearing pathogens is crucial.

Lavender EO worked synergistically with penicillin when tested against *S. agalactiae*, possibly by increasing membrane permeability or inhibiting β-lactamase, thereby enhancing the antibiotic's effectiveness (Raikwar *et al*., 2024). In contrast, mint EO had an antagonistic effect. However, both EOs showed antagonism when combined with gentamicin, which may result from competition for bacterial targets or interference with antibiotic uptake. For methicillin-resistant Staphylococcus aureus (MRSA-d), lavender EO enhanced the effects of tetracycline and gentamicin, while mint EO showed no effect with tetracycline and was antagonistic with gentamicin. The synergy between lavender EO and tetracycline may be due to its ability to disrupt bacterial membranes, making it easier for the antibiotic to penetrate (Moghrovyan and Sahakyan, 2024). On the other hand, the antagonism observed between mint EO and gentamicin suggests it may interfere with the antibiotic's uptake or activity (Aelenei *et al*., 2016). Interestingly, both EOs showed synergy with AMC and AUM, suggesting that their interactions with antibiotics can vary depending on the bacterial strain (Ellouze *et al*., 2024).

The interaction of EOs with gentamicin, chloramphenicol, AMC, AUG, and streptomycin consistently resulted in reduced inhibition zones in Gram-negative bacteria, indicating an antagonistic effect. This antagonism is probably due to the complex outer membrane structure of Gram-negative bacteria, which can limit the combined effectiveness of EOs and antibiotics (Tambe *et al*., 2023). Furthermore, the presence of efflux pumps and enzymatic degradation mechanisms in these bacteria may further reduce the effectiveness of these combinations, as noted by Başaran and Öksüz (2023). These findings highlight the need for careful evaluation of EO-antibiotic combinations, as their interactions vary greatly depending on the bacterial species and the type of antibiotic used.

CONCLUSION

Lavender essential oil demonstrates superior antimicrobial activity, particularly against drug-resistant bacteria such as MRSA. It proved more effective than mint EO, exhibiting a faster bactericidal effect and enhanced efficacy when combined with antibiotics. These findings suggest that lavender EO holds significant promise as a natural alternative in combating veterinary pathogens. In light of the growing issue of antibiotic resistance, further research into lavender EO as a potential treatment in veterinary medicine is essential.

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Competing interests

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