

Research Article

EVALUATION OF THE ANTIMICROBIAL EFFICACY OF *CUPRESSUS MACROCARPA* AND *THYMUS VULGARIS* ESSENTIAL OILS AGAINST SELECTED MULTIDRUG-RESISTANT PATHOGENS OF VETERINARY AND HUMAN ORIGIN

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Received 12 April 2025, revised 23 April 2026

ABSTRACT: This study evaluated the antimicrobial efficacy of essential oils (EOs) extracted from *Cupressus macrocarpa* (*C. macrocarpa*) and *Thymus vulgaris* (*T. vulgaris*) against selected multidrug-resistant (MDR) bacterial and fungal isolates originating from both veterinary and human sources. Using agar disc diffusion and broth dilution methods, we evaluated their potential as alternatives to conventional antimicrobials by determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC). Time-kill kinetics and antibiotic interaction assays were also conducted. Both EOs exhibited notable antimicrobial activity against the selected MDR pathogens. Notably, *T. vulgaris* demonstrated superior antimicrobial efficacy, with lower MIC (9–25 $\mu\text{L}/\text{mL}$) and MBC (12–40 $\mu\text{L}/\text{mL}$) values compared to *C. macrocarpa* (MIC: 12–40 $\mu\text{L}/\text{mL}$; MBC: 15–50 $\mu\text{L}/\text{mL}$). It also showed potent antifungal activity, achieving the lowest MFC (30 $\mu\text{L}/\text{mL}$) against yeast isolates. Time-kill assays revealed that *T. vulgaris* eradicated most pathogens within 2 hours, while *C. macrocarpa* requires up to 24 hours. Furthermore, antibiotic interaction studies indicated that *T. vulgaris* enhanced the efficacy of conventional antibiotics synergistically, whereas *C. macrocarpa* exhibited antagonistic interactions that may compromise antibiotic effectiveness. These findings underscore the therapeutic potential of *T. vulgaris* as a promising candidate for treating MDR infections and as a valuable adjunct to antibiotic therapy. However, caution is warranted when co-administering *C. macrocarpa* with antibiotics due to its antagonistic effects. Overall, this study highlights the potential role of essential oils, particularly *T. vulgaris*, in addressing antimicrobial resistance (AMR) in both veterinary and human medicine.

Keywords: Multidrug-resistant, Veterinary and human isolates, *Cupressus macrocarpa*, *Thymus vulgaris*, Time-kill assay and Required dose.

INTRODUCTION

In recent years, the search for innovative and naturally derived antimicrobial agents has intensified in response to the growing challenge of drug-resistant pathogens [1] having saved millions of lives from infectious diseases. Microbes have developed acquired antimicrobial resistance (AMR). Among these natural alternatives, essential oils have garnered significant attention due to their potent bioactive properties and broad-spectrum activity against bacteria and fungi [2]. Derived from various plant parts such as leaves, seeds, flowers, and bark, essential oils are complex mixtures of volatile aromatic compounds known for their distinctive

fragrances and multifaceted biological activities [3]. Their antimicrobial properties make them promising candidates as natural alternatives to synthetic agents

Essential oils of particular interest are Lemon Cypress oil, derived from *Cupressus macrocarpa* 'Goldcrest' [4] and thyme oil, extracted from *T. vulgaris* [5]. Both have demonstrated remarkable efficacy against a wide range of bacterial and fungal pathogens, attributed to their rich composition of bioactive compounds [6]. *C. macrocarpa* EO is characterized by its high concentration of monoterpenes, sesquiterpenes, and other volatile organic compounds, such as limonene, α -pinene, β -pinene, δ -3-carene, and myrcene, which contribute to its strong

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antimicrobial properties [7]. These compounds exert their effects by disrupting microbial cell membranes, inhibiting essential enzymes, and interfering with genetic material, ultimately leading to cell death [8].

Similarly, *T. vulgaris* EO is renowned for its antimicrobial activity, primarily due to its major bioactive components, thymol and carvacrol [9]. These phenolic monoterpenoids exhibit broad-spectrum antimicrobial effects against bacteria, fungi, and even certain viruses. Their mechanisms of action include membrane disruption, enzyme inhibition, and interference with genetic material, resulting in microbial cell death [10].

Despite the growing interest in these essential oils, a comprehensive analysis of their antimicrobial activities against clinically significant bacterial and fungal isolates from both human and veterinary sources was lacking. This study aimed to address this gap by evaluating the antibiotic sensitivity profiles of these isolates and assessing the antimicrobial efficacy of *C. macrocarpa* and *T. vulgaris* essential oils. Specifically, the study determined their minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC), and performed time-kill assays to evaluate their antimicrobial kinetics. Additionally, the study conducted a direct comparative analysis of the two essential oils, explored their potential synergistic effects with conventional antimicrobials, and assessed their cross-species applicability.

MATERIALS AND METHODS

Ethical approval

The study was approved by the Ethical Committee of the College of Veterinary Medicine, University of Duhok, Iraq (Permit number: CVM2024/0110UoD).

Study period and location

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

Tree Materials and Essential Oil Extraction

Samples of *C. macrocarpa* and *T. vulgaris* were collected from farms in Duhok Province, Iraq. The plant species were identified by a taxonomist at the University of Duhok. The samples were air-dried indoors, and essential oils (EOs) were extracted using a Clevenger apparatus, achieving a purity of $\geq 99\%$.

Antimicrobial and Essential Oil Sensitivity Profiling

Antimicrobial susceptibility testing was performed

using the disc diffusion method. Nine antibiotics were tested on animal bacterial isolates, and 13 antibiotics were tested on human isolates, along with six antifungal discs for *Candida albicans* (*C. albicans*).

Animal bacterial isolates were sourced from veterinary cases at the College of Veterinary Medicine, University of Duhok, Iraq, including *E. coli*, *Pasteurella multocida* (*P. multocida*), *Klebsiella pneumoniae* (*K. pneumoniae*), and *Proteus mirabilis* (*P. mirabilis*) from pneumonic sheep and goats [11], MRSA strains from Rasol and Abdulrahman, (2023) [12] sheep, and goats using traditional and molecular characterization. The study was conducted in Duhok province during the period from November 2021 to March 2022. Furthermore, the isolates were screened for the presence of MRSA using the standard Kirby-Bauer disk diffusion method (oxacillin discs, and *Corynebacterium pseudotuberculosis* (*C. pseudotuberculosis*) and *Streptococcus agalactiae* (*S. agalactiae*) from infected sheep and cattle [13]. Human isolates were identified using the VITEK system at Zariland Medical Laboratories/ Duhok province, Iraq.

The Kirby-Bauer method was modified by applying 15 μL of *C. macrocarpa* and *T. vulgaris* essential oils to culture plates, which were incubated at 37°C for 24-48 hours. Results were interpreted according to CLSI guidelines for both human [14] and animal isolates [15]. Isolates were classified as susceptible or resistant, with intermediate sensitivity categorized as resistant.

MIC, MBC, and MFC Determination

The MIC, MBC, and MFC of the tested EOs were determined using broth dilution testing [2]. Various EO concentrations were tested against bacterial and fungal isolates. For each, 1 ml of 5×10^6 CFU/ml bacteria or yeast was added to 1.5 ml microtubes, followed by EO. The microtubes were vortexed and incubated in a shaker incubator at 37°C for 24-48 hours. The MIC was the lowest EO concentration preventing visible growth. MBC/MFC were determined by subculturing 20 μL from MIC tubes onto MHA for bacteria (blood agar for *Corynebacterium pseudotuberculosis*) and Sabouraud Dextrose Agar (SDA) or blood agar for yeast. MBC/MFC were confirmed by the absence of growth on agar plates after incubation at 37°C for 24-48 hours.

Time-Kill Assay

The antimicrobial activity of selected essential oils (EOs) was assessed using a time-kill kinetic assay, following standardized microbiological procedures outlined by Hulankova (2024)[16]. Briefly, quantification of viable microbial cells was performed

by determining colony-forming units (CFU/mL) for bacterial isolates and *C. albicans*, using a combination of optical density (OD) measurements and two-fold serial dilution, as outlined by Mira, Yeh, and Hall (2022)[17].

Microbial cultures were grown in Mueller–Hinton Broth (MHB) under aerobic conditions at 37 °C in a shaker incubator (Forma orbital shaker Thermo, UK) at 150 rpm until reaching the logarithmic growth phase. The optical density of bacterial cultures was measured at 600 nm [18], whereas *C. albicans* suspensions were measured at 550 nm [19] (CFU, using a spectrophotometer (Libra S22, Biochrom, Scientific Laboratory Suppliers, UK). OD–CFU calibration curves were generated to determine CFU/mL and to prepare standardized challenge inocula. For inoculum preparation, 1 mL of a culture adjusted to 1×10^8 CFU/mL was transferred into sterile 15 mL centrifuge tubes and centrifuged at $1000 \times g$ for 10 min. Supernatants were discarded, and the pellets were resuspended in sterile phosphate-buffered saline (PBS) to obtain a final concentration of 5×10^6 CFU/mL. Microbial suspensions (5×10^6 CFU/mL) were treated with EO at MBC/MFC concentrations and incubated at 37°C with shaking. Aliquots were taken at 2, 4, and 24 hours, plated onto agar, and incubated to determine viable counts. Controls without EO were included.

Synergistic and Antagonistic Effects of EO and Antibiotics:

The study investigated the combined effects of essential oils (EOs) from *C. macrocarpa* and *T. vulgaris* with antibiotics or antifungals on bacterial and yeast isolates to assess interactions with antimicrobials that did not individually reach the sensitivity threshold. A 6 µl concentration of EO ($0.5 \times \text{MIC}$) was used to evaluate synergistic or antagonistic effects. Bacterial and fungal cultures were grown on Mueller-Hinton and blood agar, with antibiotic discs saturated with EOs (6 µl)[20]. Synergy was indicated by an increase of >2 mm in the inhibition zone compared to either antimicrobial or EO alone [21]. Antagonism was observed if the inhibition zone was reduced by >2 mm when used in combination [22]. 0.5, 1, and 2 µg/ml of vancomycin. and the zones of inhibition were measured at 24 h. The CLSI-recommended checkerboard method was used as a reference to detect synergy.

Statistical Analysis

Significant differences in inhibition zones between EOs and effective antibiotics against veterinary

bacterial strains were analyzed using one-way ANOVA in GraphPad Prism 8.0.1. Additionally, Fisher's Exact Test was applied to assess the antibacterial efficacy of *C. macrocarpa* and *T. vulgaris* at 2 and 4 hours post-treatment. Results were presented as mean \pm SE from three independent experiments, with statistical significance set at $p < 0.05$.

RESULTS

Susceptibility patterns to antibiotics, antifungals, and essential oils:

The antibiotic susceptibility testing of veterinary isolates revealed widespread resistance to penicillin and tetracycline, with all isolates except *C. pseudotuberculosis* showing resistance (Table 1). Methicillin-resistant *Staphylococcus aureus* (MRSA) strains (CTI, D, and C) and *E. coli* were resistant to streptomycin, while *C. pseudotuberculosis* and *S. agalactiae* remained susceptible. *P. multocida* exhibited resistance to chloramphenicol. Amoxicillin-clavulanic acid demonstrated variable efficacy, with resistance observed in *P. multocida*, *P. mirabilis*, and MRSA-D and MRSA-C. The emergence of multidrug resistance in veterinary clinical isolates is further evidenced by the prevalence of ceftiofur resistance in *E. coli* and MRSA-C, as well as sulfamethoxazole-trimethoprim resistance in *E. coli*, *P. mirabilis*, and *Streptococcus agalactiae*. In contrast, *S. agalactiae* and *K. pneumoniae* remained highly susceptible to sulfamethoxazole-trimethoprim. These findings underscore the need for targeted antibiotic use in veterinary medicine.

The essential oils of *C. macrocarpa* and *T. vulgaris* demonstrated potent antibacterial activity against all tested veterinary isolates, including MRSA strains (Table 1). The inhibition zones ranged from 26.7 ± 0.9 mm to 34.0 ± 0.6 mm, with the most pronounced activity observed against the MRSA- CTI isolate (*C. macrocarpa*: 34.0 ± 0.6 mm; *T. vulgaris*: 32.0 ± 2.1 mm). This indicates a strong inhibitory effect of both EOs, particularly against resistant Gram-positive pathogens.

Human bacterial and fungal isolates displayed varying resistance patterns. *E. coli* and *K. pneumoniae* were resistant to ampicillin, cefepime, and ceftazidime, while *S. aureus* and *S. haemolyticus* exhibited variable resistance profiles. Ciprofloxacin and gentamicin were effective against *E. coli*, *E. faecalis*, and *S. aureus* but showed limited efficacy against *K. pneumoniae* and *S. haemolyticus*. Tigecycline demonstrated broad susceptibility across isolates, whereas vancomycin resistance was observed in multiple strains. Antifungal

testing of *C. albicans* revealed resistance to azole such as itraconazole, fluconazole, ketoconazole, and miconazole, raises significant concerns for the future of antifungal therapy, though amphotericin B and nystatin remained effective (Table 2).

Similarly, both essential oils exhibited substantial antibacterial and antifungal activity against human clinical isolates. In this group, *C. macrocarpa* produced inhibition zones ranging from 22.7 ± 2.9 mm to 28.1 ± 0.1 mm, while *T. vulgaris* showed slightly higher zones of inhibition, ranging from 27.7 ± 0.3 mm to 28.3 ± 0.9 mm (Table 2).

Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), and Minimum Fungicidal Concentration (MFC)

The MIC, MBC, and MFC values varied depending on the essential oil used. *T. vulgaris* consistently exhibited lower MIC and MBC values compared to *C. macrocarpa*, indicating stronger antibacterial activity. Similarly, *T. vulgaris* demonstrated greater antifungal efficacy, as evidenced by lower MFC values against yeast isolates (Table 3). The lowest MIC and MBC values for *T. vulgaris* were observed against *E. coli* and MRSA (*S. aureus*), with values as low as 9–12 $\mu\text{L}/\text{mL}$. In contrast, *C. macrocarpa* required higher concentrations (12–15 $\mu\text{L}/\text{mL}$) for the same isolates.

Time-Kill Assay at MBC/MFC

The time-kill assay demonstrated that *T. vulgaris* EO exhibited a faster and more potent bactericidal and fungicidal effect compared to *C. macrocarpa*. Within 2 hours of treatment, *T. vulgaris* eliminated all bacterial and fungal isolates except *P. mirabilis*, *E. faecalis* and *C. albicans*. In contrast, *C. macrocarpa* only eradicated *K. pneumoniae* and MRSA-C.

Fisher's Exact Test revealed a significant difference in the antimicrobial efficacy between the two oils. At 2 hours, the odds ratio was 0.033 ($p = 0.00024$), indicating that *C. macrocarpa* was significantly less effective than *T. vulgaris* in inhibiting bacterial growth. After 4 hours, *T. vulgaris* achieved complete inhibition of bacterial growth in all tested isolates, whereas some isolates remained viable when treated with *C. macrocarpa* (odds ratio = 0.0, $p = 0.0177$). By 24 hours, *C. macrocarpa* also achieved complete inhibition of all isolates (Table 4). These findings underscore the strong antimicrobial potential of *T. vulgaris* as a viable alternative therapeutic agent for tackling multidrug-resistant infections where conventional treatments fail.

Interactions Between Essential Oils and Antibiotics

The study also assessed the interaction between antibiotics and the EOs of *C. macrocarpa* and *T. vulgaris* against various bacterial isolates (Table 5). *T. vulgaris* consistently exhibited synergistic effects, enhancing antibiotic efficacy, while *C. macrocarpa* showed antagonistic interactions when combined with chloramphenicol, streptomycin, amoxicillin-clavulanic acid (AMC/AUG), and penicillin against *P. multocida*, *P. mirabilis*, *K. pneumoniae*, and MRSA-C and MRSA-D, resulting in reduced inhibition zones. Additionally, an indifferent effect was observed when *C. macrocarpa* was combined with azithromycin against MRSA-D, indicating no significant enhancement in antimicrobial activity.

DISCUSSION

The findings of this study highlight the alarming prevalence of antimicrobial resistance in bacterial isolates from both animals and humans, underscoring the urgent need for robust antimicrobial stewardship programs and the development of alternative treatments. The antibiotic susceptibility testing of veterinary isolates revealed widespread resistance to penicillin and tetracycline. The widespread resistance to commonly used antibiotics, such as penicillin and tetracycline, observed in animal isolates, reflects the declining efficacy of these drugs [23, 24]. Notably, *E. coli*, *P. mirabilis*, and MRSA exhibited resistance to streptomycin, while *P. mirabilis*, MRSA, and *P. multocida* showed resistance to amoxicillin-clavulanic acid. This resistance is likely driven by the improper use of antibiotics by local farmers, who often store and administer drugs like oxytetracycline, tylosin, streptomycin, and penicillin without veterinary oversight. Such practices, including underdosing, overuse, and premature cessation of treatment, facilitate bacterial adaptation through mechanisms such as aminoglycoside-modifying enzymes, ribosomal mutations, and efflux pumps, ultimately reducing antibiotic efficacy [25]. The prominent cause contributing to the current crisis remains to be the overuse and misuse of antimicrobials, particularly the inappropriate usage of antibiotics, increasing the global burden of antimicrobial resistance. The global consumption and usage of antibiotics are therefore closely monitored at all times. Additionally, resistance to amoxicillin-clavulanic acid in *P. mirabilis* and MRSA is primarily attributed to β -lactamase production, which degrades the antibiotic despite the presence of clavulanic acid [26].

Table 1: Antibiotic sensitivity profiles of veterinary isolates, highlighting resistance and susceptibility patterns across various bacterial strains.

Antimicrobials	<i>E. coli</i>	<i>P. multocida</i>	<i>P. mirabilis</i>	<i>K.pneumonia</i>	<i>C. pseudotuberculosis</i>	<i>Streptagalactiae</i>	<i>MRSA-CTI</i> (Cattle isolate)	<i>MRSA-CTII</i> (Dog isolate)	<i>MRSA-C</i> (Cat isolate)
Penicillin	R	R	R	R	S	R	R	R	R
	0	6.3 ± 0.3	4.0 ± 0.6	13.0 ± 1.5	30.7 ± 1.2	10.3 ± 0.3	0	0	0
Tetracycline (TE)	R	R	R	R	S	R	R	R	R
	5.3 ± 0.3	0	0	0	26.7 ± 3.3	0	9.0 ± 0.6	11.3 ± 3.0	6.7 ± 1.3
	R ^{Cu** T**}	S ^{Cu***}	R	S ^{CuT}	S	S ^{Cu** T**}	R	S ^{Cu*** T***}	R
Streptomycin (S)	15.5 ± 0.5	16.3 ± 2.6	14 ± 1.2	17.0 ± 1.5	25.7 ± 3	20.3 ± 1.5	9.0 ± 0.6	15.0 ± 0.6	10.0 ± 1.0
	S	R	R	S	S	S	S ^{Cu*** T**}	S ^{Cu** T**}	S ^{Cu*** T**}
Chloramphenicol (C)	27.5 ± 2.5	12.3 ± 0.3	12.3 ± 0.3	30.0 ± 1.7	28.3 ± 1.7	22.7 ± 0.3	23.3 ± 0.9	24.7 ± 2.7	21.0 ± 0.6
	S	R	R	S	S	S	S	S	R
AMC or AUG	23 ± 2.5	16.7 ± 0.7	16.7 ± 0.7	24.3 ± 1.2	25.0 ± 2.9	21.0 ± 0.6	30.3 ± 0.3	30.7 ± 0.7	13.0 ± 0.6
	R	S	S	S	S	S ^{Cu* T*}	S ^{Cu* T*}	S	R
Ceftiofur (CFT)30µg	6 ± 0.6	26.0 ± 3.1	23.5 ± 0.5	27.5 ± 2.5	31.5 ± 1.5	27.3 ± 1.5	26.0 ± 0.6	30.7 ± 0.7	23.0 ± 1.2
	R	S	R	S	S	R	S ^{Cu*}	S	S ^{Cu* T*}
SMX-TMP	0	27.0 ± 1.5	0	29.5 ± 2.5	29.3 ± 0.7	0	27.3 ± 1.5	28.3 ± 1.7	25.7 ± 1.3
	R	S	S ^{CuT}	S	S	R	R	R	R
AZM	1.3 ± 0.7	23.3 ± 2.0	19.3 ± 1.8	23.0 ± 2.0	27.3 ± 1.5	0	4.7 ± 2.9	7. ± 1.5	10. ± 2.9
	R	R	R	R	S	R	R	S	R
Tylosin 30µg	0	0	0	0	35.7 ± 4.7	7.3 ± 3.7	3.3 ± 1.7	27.3 ± 1.5	13.5 ± 1.5
	S	S	S	S	S	S	S	S	S
<i>Cupressus macrocarpa</i>	28.7 ± 0.7	29.7 ± 0.9	28.7 ± 0.7	28.7 ± 0.7	26.7 ± 0.9	27.7 ± 0.3	34.0 ± 0.6	31.3 ± 1.2	26.7 ± 0.9
	S	S	S	S	S	S	S	S	S
<i>Thyme vulgaris</i>	27.7 ± 1.5	28.3 ± 0.9	27.3 ± 0.7	28.7 ± 0.7	27.0 ± 0.6	27.7 ± 0.3	32.0 ± 2.1	31.7 ± 0.9	27.0 ± 0.6
	R	R	R	R	R	R	R	R	R

The superscripts “Cu” and “T” indicate significant differences in the inhibitory zones (in mm) between the essential oils (EOs) of *Cupressus macrocarpa* and *Thymus vulgaris*, as well as antibiotics. Asterisk notations (*, **, ***) correspond to the following levels of significance: p < 0.05, p < 0.01, and p < 0.001, respectively.

Table 2: Antibiotic and antifungal sensitivity profiles of human-origin bacterial and fungal isolates, with resistance (R) and susceptibility (S) observed across various agents.

Antibiotics	Bacterial and fungal isolates of human origin					
	<i>E. coli</i>	<i>E. faecalis</i>	<i>K. pneumoniae</i>	<i>S. aureus</i>	<i>S. haemolyticus</i>	<i>C. albican</i>
Ampicillin	R	-	R	-	-	-
Cefepime	R	-	R	R	R	-
Ceftazidime	R	-	R	-	R	-
Ciprofloxacin	S	S	R	S	R	-
Clindamycin	-	S	-	S	R	-
Erythromycin	R	R	-	-	R	-
Gentamicin	S	S	S	R	S	-
Imipenem	S	-	S	-	-	-
Meropenem	S	-	S	-	-	-
Tetracycline	S	R	R	S	R	-
Tigecycline	S	S	S	S	S	-
(SMX-TMP)	S	-	R	S	S	-
Vancomycin	-	R	-	R	R	-
Itraconazole	-	-	-	-	-	R
Amphotericin B	-	-	-	-	-	S
Fluconazole	-	-	-	-	-	R
Ketoconazole	-	-	-	-	-	R
Nystatin (NS)	-	-	-	-	-	S
Miconazole	-	-	-	-	-	R
<i>Cupressus macrocarpa</i>	S 28.1 ± 0.1	S 26.± 2	S 26± 2	S 22.7 ±2.9	S 26.7 ± 3.5	S 26. ± 3.
<i>Thyme vulgaris</i>	S 28.2 ± 0.2	S 28.3 ± 0.3	S 27.7 ± 0.3	S 28.3 ±0.3	S 28.3 ± 0.9	S 27.7 ± 0.3

The emergence of multidrug resistance in veterinary clinical isolates is further evidenced by the prevalence of ceftiofur resistance in *E. coli* and MRSA-C, as well as sulfamethoxazole-trimethoprim resistance in *E. coli*, *P. mirabilis*, and *Streptococcus agalactiae*. The high resistance to azithromycin in *E. coli* and MRSA, coupled with the limited efficacy of tylosin, underscores the escalating challenge of AMR in veterinary medicine [26]. These findings emphasize the need for ongoing surveillance and judicious antibiotic selection in veterinary practices [27].

Human bacterial and fungal isolates displayed varying resistance patterns. Concerning resistance patterns, with *E. coli* and *K. pneumonia* showing significant resistance to ampicillin, cefepime, and ceftazidime, complicating treatment options [28]. Similarly, diverse resistance patterns in *S. aureus* and *S. haemolyticus* highlight the importance of personalized antibiotic therapy based on specific resistance profiles [29]. While ciprofloxacin

and gentamicin remain effective against *E. coli*, *E. faecalis*, and *S. aureus*, their limited efficacy against *K. pneumoniae* and *S. haemolyticus* underscores the need for targeted antimicrobial strategies [30]. Tigecycline demonstrated broad susceptibility, consistent with findings by Kenza El Bazi *et al.*, (2022) [26]. However, widespread vancomycin resistance across multiple bacterial strains raises serious concerns for managing severe infections, necessitating continuous monitoring and adaptable treatment protocols [32]. Addressing these challenges requires a multifaceted approach, including the development of novel antibiotics, strengthened infection control measures, and robust antimicrobial stewardship programs [33].

The high resistance of *Candida albicans* to azole antifungals, such as itraconazole, fluconazole, ketoconazole, and miconazole, raises significant concerns for the future of antifungal therapy. While amphotericin B and nystatin retain efficacy, the increasing prevalence

Table 3: Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values for bacterial and fungal isolates of animal and human origin against *Cupressus macrocarpa* and *Thyme vulgaris*.

Bacterial Isolates-Animal Origion	<i>Cupressus macrocarpa</i>		<i>Thyme vulgaris</i>	
	MIC	MBC	MIC	MBC
<i>Escherichia coli</i>	12	15	9	12
<i>P. multocida</i>	12	15	12	15
<i>P. mirabilis</i>	40	50	25	40
<i>K.pneumonia</i>	12	12	12	15
<i>C. pseudotuberculosis</i> (Sheep isolate)	15	18	15	18
<i>Streptococcus agalactiae</i>	40	50	15	20
<i>Staph aureus</i> MRSA CTI (Cattle isolate)	12	15	9	12
<i>Staph aureus</i> MRSA CTII (Cattle isolate)	12	15	9	12
<i>Staph aureus</i> MRSA D (Dog isolate)	12	15	9	12
<i>Staph aureus</i> MRSA C (Cat isolate)	12	15	9	12
Bacterial and fungal Isolates-human Origion				
<i>Escherichia coli</i>	12	15	9	12
<i>K.pneumonia</i>	12	15	9	12
<i>Staphylococcus aureus</i>	12	15	9	12
<i>Staphylococcus haemolyticus</i>	12	15	9	12
<i>Enterococcus faecalis</i>	35	50	15	18
<i>Candida albicans</i>	30	40	15	30

Table 4: Time required to kill bacterial and fungal isolates at the minimum bactericidal concentration (MBC) for various bacterial isolates, using *Cupressus macrocarpa* and *Thyme vulgaris*. 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
	<i>C. macrocarpa</i>	<i>T. vulgaris</i>	<i>C. macrocarpa</i>	<i>T. vulgaris</i>	<i>C. macrocarpa</i>
Animal Isolates					
<i>E. coli</i>	G	NG	G		NG
<i>K. pneumoniae</i>	NG	NG	-		
<i>P. multocida</i>	G	NG	NG		
<i>P. mirabilis</i>	G	G	NG	NG	
<i>C. pseudotuberculosis</i>	G	NG	NG		
<i>S. agalactiae</i>	G	NG	G		NG
<i>Staph aureus</i> MRSA CTI (Cattle isolate)	G	NG	G		NG
<i>Staph aureus</i> MRSA CTII (Cattle isolate)	G	NG	G		NG
<i>Staph aureus</i> MRSA D (Dog isolate)	G	NG	NG		
<i>Staph aureus</i> MRSA C (Cat isolate)	NG	NG	-		-
Human Isolates					
<i>E.coli</i>	G	NG	NG		
<i>K. pneumoniae</i>	G	NG	G		NG
<i>Staphylococcus aureus</i>	G	NG	NG		
<i>Staphylococcus hamolyticus</i>	G	NG	G		NG
<i>Enterococcus faecalis</i>	G	G	NG	NG	
<i>Candida albicans</i>	G	G	NG	NG	

G = growth and NG = no growth

Table 5: Antimicrobial activity of *Cupressus macrocarpa* and *Thyme vulgaris*, alone and in combination with antibiotics, against bacterial isolates from animals. The inhibition zone diameters (mm) are presented as mean \pm standard error. 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.

Bacteria-Animal Isolates	Antibiotic	Alone mm	<i>Cupressus macrocarpa</i>			<i>Thyme vulgaris</i>		
			Alone mm	Combination mm	results	Alone mm	Combination mm	results
<i>P. multocida</i>	Chloramphenicol	12.3 \pm 0.3		21.5 \pm 1.5	A		36.5 \pm 1.5	S
	AMC or AUG	16.7 \pm 0.7	29.7 \pm 0.9	16.5 \pm 1.5	A	28.3 \pm 0.9	31.5 \pm 1.5	S
<i>P. mirabilis</i>	Streptomycin	14 \pm 1.2		17.5 \pm 0.5	A		35.5 \pm 2.5	S
	AMC or AUG	16.7 \pm 0.7	28.7 \pm 0.7	18	A	27.3 \pm 0.7	36 \pm 2	S
<i>K. pneumoniae</i>	Penicillin	13.0 \pm 1.5	28.7 \pm 0.7	11 \pm 1	A	28.7 \pm 0.7	37.5 \pm 2.5	S
<i>S. agalactiae</i>	Penicillin	10.3 \pm 0.3	27.7 \pm 0.3	37.5 \pm 0.5	S	27.7 \pm 0.3	38.6 \pm 2.3	S
MRSA CTII (Cattle isolate)	Tetracycline	11.3 \pm 3.0	31.3 \pm 1.2	35.5 \pm 2.5	S	31.7 \pm 0.9	34+1	S
	Tetracycline	13.3 \pm 0.9		29.5 \pm 0.5	S		36.5 \pm 1.5	S
MRSA-D (Dog isolate)	Streptomycin	15.0 \pm 0.6	26.7 \pm 0.9	23 \pm 0.1	A	27.0 \pm 0.6	38	S
	Azithromycin	10. \pm 2.9		27.5	I		38	S
MRSA- C (Cat isolate)	Streptomycin	10.0 \pm 1.0		16 \pm 1	A		35 \pm 2.5	S
	AMC or AUG	13.0 \pm 0.6	32.0 \pm 0.6	24 \pm 1	A	27.7 \pm 0.3	31.5 \pm 1.5	S

of azole-resistant strains underscores the need for novel antifungal compounds [34].

The routine use of antibiotics in animals for growth promotion, disease prevention, and treatment drives bacterial adaptation, leading to the emergence of resistant pathogens [25]. These resistant bacteria can transfer to humans through direct contact, contaminated food, or environmental exposure, complicating treatment options [35]. The widespread use of antibiotics in food-producing animals exerts selective pressure, promoting the persistence and spread of resistant strains, which are then transmitted to humans [36]. This highlights the urgent need for enhanced antimicrobial stewardship and surveillance to curb the transfer of resistance from animal reservoirs to human clinical settings [32, 33]

The essential oils of *C. macrocarpa* and *T. vulgaris* exhibit potent antibacterial activity against multidrug-resistant isolates, including MRSA, from both veterinary and human sources, demonstrating their potential as alternative antimicrobial agents. These findings are consistent with earlier reports suggesting the broad-spectrum efficacy of plant-derived EOs against resistant pathogens [2, 34]. *T. vulgaris* demonstrated superior efficacy compared to *C. macrocarpa*, with pronounced inhibition of MRSA likely due to mechanisms such as membrane disruption and metabolic interference [40].

The antimicrobial efficacy of these essential oils was further confirmed by MIC, MBC, and MFC assessments. *T. vulgaris* exhibited lower MIC and MBC values, indicating potent bactericidal properties, as well as reduced MFC values, suggesting enhanced fungicidal activity [41].

Time-kill assays at MBC/MFC concentrations revealed that *T. vulgaris* exerted rapid and potent bactericidal and fungicidal effects, eliminating most isolates within two hours. In contrast, *C. macrocarpa* exhibited slower antimicrobial activity, with complete inhibition observed only after 24 hours. These findings underscore the strong antimicrobial potential of *T. vulgaris* as a viable alternative therapeutic agent for tackling multidrug-resistant infections where conventional treatments fail.

This study also highlights the complex interactions between essential oils and antibiotics. *T. vulgaris* consistently exhibited synergistic effects, enhancing antibiotic efficacy, while *C. macrocarpa* showed antagonistic interactions. These contrasting outcomes are likely due to differences in their chemical composition. The high levels of thymol and carvacrol in *T. vulgaris* may enhance membrane disruption and antibiotic uptake [37, 38]. In contrast, antagonism observed with *C. macrocarpa* may result from compounds that interfere with antibiotic permeability

or alter bacterial metabolism [44]. Thus, while *T. vulgaris* holds promise as an antibiotic adjuvant, caution is warranted when combining *C. macrocarpa* with conventional antimicrobials. Further studies are needed to clarify the mechanisms underlying these interactions.

In conclusion, this study underscores the growing threat of antibiotic resistance, particularly against commonly used antibiotics, with multidrug-resistant strains posing significant challenges. *T. vulgaris* essential oil demonstrated strong antimicrobial activity against multidrug-resistant bacterial and fungal pathogens from both veterinary and human sources, exhibiting superior efficacy (lower MIC, MBC, and MFC values) and rapid killing kinetics. Its synergistic interactions with antibiotics suggest its potential as an adjunct therapy for multidrug-resistant infections. While *C. macrocarpa* also showed antimicrobial activity, its antagonistic effects with certain antibiotics warrant caution in combination treatments. Overall, *T. vulgaris* holds promise as a natural antimicrobial agent in combating resistance in clinical and veterinary medicine.

Authors contribution: Renas Ihsan Abdulkarim and Nawzat Aboziad Issa conceived and designed the study. NAI and RIA executed the experiment and analysed the data. All authors interpreted the data, critically revised the manuscript for important intellectual contents and approved the final version.

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Cite this article as: Abdulkarim RI and Issa NA. Evaluation of the antimicrobial efficacy of *Cupressus macrocarpa* and *Thymus vulgaris* essential oils against selected multidrug-resistant pathogens of veterinary and human origin. *Explor Anim Med Res.* 2026; 16(1), DOI: 10.52635/eamr/16.1.52-62.