Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
DOI: 10.29261/pakvetj/2024.165

RESEARCH ARTICLE

Evaluation the Antimicrobial Activity of Essential Oils against Veterinary Pathogens, Multidrug-resistant Bacteria and Dermatophytes

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ARTICLE HISTORY (24-047)

Received: January 29, 2024
Revised: April 4, 2024
Accepted: April 6, 2024
Published online: April 28, 2024

Key words:
Antimicrobial activity
Essential oils
Veterinary pathogens

ABSTRACT

This study aimed to determine the antibiotic and antifungal susceptibility profiles of animal clinical bacterial and fungal isolates and to evaluate the antimicrobial activities of essential oils (EOs) in both the agar disc diffusion method and the broth dilution assay. The minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of thyme, mint, and lavender EOs were evaluated. The results of the antibiotic and antifungal susceptibility profiles tests showed differences in the bacterial sensitivities to the studied antibiotics and antimycotics with the emerging of multidrug-resistant bacteria and dermatophytes. Ciprofloxacin was the most effective antibiotic and the tested fungal isolates were much more sensitive to ketoconazole than other antifungals. Thyme essential oil exhibited potent antibacterial activity against every tested strains of bacteria with MICs of less than 9µl/ml (0.9%) for the majority of the tested pathogens. The tested EOs effectively inhibited the growth of dermatophytes. *Thyme* oil presents itself as a promising antibacterial and anti-fungal agent against veterinary pathogens, being a natural product that can represent an interesting antimicrobial in the efforts to combat bacterial and fungal infections in veterinary medicine.

To Cite This Article: Issa NA, 2024. Evaluation the antimicrobial activity of essential oils against veterinary pathogens, multidrug-resistant bacteria and dermatophytes. Pak Vet J, 44(2): 260-265. http://dx.doi.org/10.29261/pakvetj/2024.165

INTRODUCTION

The last several years have seen a noticeable rise in the search for novel, safe natural antimicrobial compounds, particularly those derived from plants (Pinto *et al.*, 2023). The emergence of drug-resistant bacteria is one of the main challenges to the efficient treatment of microbial illnesses (Rossolini *et al.*, 2014). Interest in plant extracts, including essential oils, has increased as a source of natural products (Bolouri *et al.*, 2022). Essential oils, often referred to as volatile oils, are aromatic, viscous liquids that are extracted from a variety of plant parts, such as leaves, twigs, fruits, bark, roots, buds, seeds, and flowers (Konfo *et al.*, 2023). Essential oils have been utilized historically for their antimicrobial properties (Ghavam *et al.*, 2022).

Thyme, lavender, and mint EOs contain various compounds with antimicrobial activities. The main components of thyme include 20–40% thymol, p-cymene and γ-terpinene that are the main phenolic components, along with, caryophyllene, terpinolene, β-myrcene, and borneol, cineol, linalool, menthone, B-cymene, pinene, and triterpenic acid (Thosar *et al*., 2013; Dong *et al*., 2023). As the primary active component that gives thyme EO its potency, thymol has been demonstrated to have antiseptic and antimicrobial characteristics (Tohidi *et al.*, 2020). Lavender essential oil consists primarily of monoterpeneoids and sesquiterpeneoids; of these, linalool and linalyl acetate dominate, with moderate levels of E-β-ocimene, terpinen-4-ol, caryophyllene, carvacrol, lavandulyl acetate, Z-β-farnesene, Z-β-ocimene and camphor are also present in low to moderate qualities. (Pokajewicz *et al.*, 2021; Kozuharova *et al.*, 2023). Studies have used lavender EO as antifungal (Zuzarte *et al.*, 2011), antibacterial (Kwiatkowski *et al.*, 2020) and antiviral (Abou Baker *et al.*, 2021). While the primary components of mint include monoterpenic alcohols, mainly menthol (38–48%), ketones, mainly menthones (20–30%) and 1,8-cineole, menthyl acetate and isovalerate, pinene, limonene and other constituents some monoterpenes, and oxides (Thosar *et al.*, 2013), it works well as an antiviral, antibacterial, and antiseptic (Chouhan *et al.*, 2017).

The efficacy of EOs in treating infections in animals is not well understood. Despite the fact that their *in vitro* antibacterial activity has been regularly shown in investigations conducted on bacterial and fungal strains of various sources (Ebani and Mancianti, 2020). Therefore,

this study aimed to investigate the *in vitro* antimicrobial efficacy of three essential oils against animal clinical bacterial and fungal isolates to determine their minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC).

MATERIALS AND METHODS

Ethical approval: The University of Duhok, Iraq's College of Veterinary Medicine's Ethical Committee gave its clearance for the study to be carried out (Permit number: VM2023/0401UD).

Study period and location: This study was conducted from January 2023 to January 2024 at the College of Veterinary Medicine, University of Duhok, Iraq.

Plant materials: Thyme, mint, and lavender were collected from independent farms in Duhok province, Iraq, and authenticated by a taxonomist at the University of Duhok's College of Agricultural Engineering Sciences. The plants were cleaned and air-dried indoors, and essential oils were extracted using a Clevenger apparatus. The purity of the extracted oils was checked and estimated to be over 99%.

Antibiotic and antifungal discs: The study tested eight antibiotic discs on Mueller Hinton Agar against bacteria and six antifungal discs on Sabouraud Dextrose Agar against dermatophytes. The antibiotics included doxycycline, erythromycin, gentamicin, ciprofloxacin, ceftriaxone, imipenem, norfloxacin, and trimethoprim-sulfamethoxazole, while the antifungal discs included Iitraconazole, Amphotericin, Fluconazole, Ketoconazole, Nystatin, and Miconazole. The isolates were classified as susceptible or resistant, with resistant isolates being intermediately sensitive to a particular antibiotic.

Determination of the antibiotic, antifungal and EOs sensitivity profile: The Kirby-Bauer technique was used to evaluate the sensitivity of the used microorganisms to antimicrobial drugs and essential oils (EOs) with a little modification. Antibiotic-containing discs were replaced with pure thyme, mint, and lavender oils (10 µl). Cultures of bacterial seeded on MHA were incubated at 37°C for 24-48hours, while fungal isolates seeded on SDA agar were incubated at 30°C for four weeks. Observations were recorded and checked.

The Clinical and Laboratory Standards Institute (CLSI, 2015) was followed in the protocol and result interpretations (break-pinots). The isolates were classified as susceptible or resistant (it was decided to classify as resistant isolates those that were intermediately sensitive to a particular antibiotic).

Bacterial and fungal suspensions: The used bacteria and fungi were isolated from veterinary clinical cases and molecularly identified at the college of the Veterinary Medicine- University of Duhok, Iraq. *Mannheimia haemolytica, pasteurella multocida, Klebsiella pneumonia, Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated from sheep slaughtered at slaughter houses in Duhok province (Ahmed and Abdullah, 2022), Methicilline resistant Staphylococcus aureus was provided by (Rasol and Abdulrahman, 2023), *Salmonella enterica* serovar newport isolated from frozen chicken carcasses (Taha *et al*., 2015), *Escherichia coli* was isolated from food products in Duhok province (Taha and Yassin, 2019). *Microsporum canis* (ON209159) and *Trichophyton mentagrophytes* (ON221385) were isolated from clinically infected cats and dogs with dermatophytosis (Jarjees and Issa, 2022) and *Corynebacterium pseudotuberculosis* (Sheep isolate (ON142642) and goat isolate (ON142653)) were isolated from clinically infected sheep and goats with caseous lympadentis (Khanamir *et al.*, 2023).

The evaluation tests involved determining the colony-forming units of bacteria and dermatophytes using serial dilution/viable colony count and spectrophotometric methods (Miles *et al*., 1938). Growths were grown in brain-heart infusion broth (Khan *et al.*, 2006) and incubated in a shaker incubator. Challenge doses of 5 x 10^6 CFU/ml were determined using a calibration curve between log10 counts and optical density.

Determination of MIC, MBC and MFC of Eos: The study used broth dilution testing (Boardman and Smith, 2016) with some modifications. Seven different concentrations of each prepared EO against bacterial and fungal isolates individually were tested. 1 ml of 5x10^6 CFU/ml of the bacteria and fungi were dispensed into 1.5 ml microtubes, followed by EO addition. The microtubes were vortexed well before being incubated at 37°C for 24 hours for bacteria and four days at 30°C for fungi. The MIC of each tested EO that prevented organisms from growing visibly in tubes was determined. The MBC/MFC were identified by sub culturing 50 µl of suspensions from MIC tubes and the one next to it onto MHA for bacteria and the fungi on SDA agar. The MBC/MFC concentrations were determined when negative microbial growth was found on the surface of agar plates after 24-48 hours of incubation at 37ºC for bacteria and four weeks for fungi at 30ºC after culturing.

Statistical analysis: The study utilized GraphPad Prism 8.0.1 software for statistical analysis, employing one-way ANOVA to detect significant differences among tested antibiotics, antifungal, and EOs. Data were presented as mean ± SE of three independent experiments, with p values <0.05 considered significant.

RESULTS

Antibiotics, antifungal and EOs susceptibility results: The results of antibiotic susceptibility tests are presented in Table 1. Differences were found in the bacterial sensitivities to the studied antibiotics, where all of the bacterial isolates were sensitive to Ciprofloxacin. Imipenem was also effective against the used isolates except *E. coli* (EHEC). Whereas, the isolates were resistant to Ceftriaxone, and resistant to Gentamicin (except *S. aureus*). On the other hand, the data revealed that the tested essential oils had broad bactericidal activities, namely thyme EO that inhibited the growth of all the tested bacteria with a large inhibitory zone ranging from 26–35 millimeter (Table 1). Lavender EO effectiveness varied with bacterial

Table 1: Antibiotic resistant profile of the used bacterial isolates in this study compared to antimicrobial activities of EOs, Thyme, Lavender and Mint.

Bcaterial Isolates	Thyme 10 µl	Mint 10 µl	Lavender 10 µl	Imipenem 10 µg	Trimethoprim-sulfamethoxazole 75 µg	Erythromycin 10 µg	Ciprofloxacin 5 µg	Gentamicin 10 µg	Norfloxacin 30 µg	Doxycycline 10 µg	Ceftriaxone 30 µg
	Inhibition Zone diameter millimeter (mm)										
E. coli (EHEC)	S***	R	S	R	S	R	S	R	S	R	R
	34.3±0.6	11±1	17.3±1	7.7±0.6	18±1	5.7±0.6	18.3±0.6	7±1	16±1	7±1	6.3±1.5
S. newport	S***	S	S	S	R	R	S	R	S	R	R
	35.3±0.6	17±2	16±1	18.7±1.5	12.7±1.5	2.7±0.6	20.7±5.5	6±2.6	16±1	4.7±2.5	7±1
S. aureus	S***	S***	S***	S***	S	R	S	S	S	S	R
	34.7±0.6	35.3±0.6	34.7±0.6	35.3±0.6	22±1	11.7±0.6	18.3±0.6	16.7±1.2	23±01	23±1	9±1
P. aeruginosa	S	R	R	S	R	R	S	R	S	R	R
	28±0.7	9±6.6	3.3±1.5	22.3±3.2	2.3±0.6	2	21±7	8.7±3.1	22.3±6.7	5±1.4	5±1
Staphylococcus aureus (MRSA)	S***	S	S	S***	R	R	S	R	R	R	R
	35.7±0.6	17±1	17.3±0.6	35	11.7±0.6	11.3±0.6	21±1	2.7±0.6	13±1	11.7±1.5	8.7±0.6
K. pneumoniae	S	S	S	S	R	R	S	R	S	R	R
	27±2.6	20	27.7±0.6	21.7±7.2	7.7±0.6	2.7±0.6	20.3±8	5.5±0.7	22.7±6.4	6.7±1.5	14.3±1.2
P. multocida	S***	R	S	S	S	R	S	R	R	S	R
	25.7±3.8	13.3±1.5	16±2.6	23.7±5.5	18±1	08±1	23.7±5.5	5±2.6	4±1.4	17.3±1.2	7±1
M. haemolytica	S	S	S	S	R	R	S	R	R	R	R
	26.3±7.6	16.3±2	19±2.6	22±2.6	8.3±0.6	5.3±0.6	23.7±5.5	6.3±3.8	6±2	3.3±1.2	6±1
C. pseudotuberculosis STS	S***	R	R	S	R	S	S	R	S	R	R
	31.3±1.2	4.3±1	4.3±1.5	19±1	2.7±0.6	22.3±0.6	25.7±0.6	2.7±0.6	18.7±0.6	10.7±1.2	2.3±0.6
C. pseudotuberculosis STG	S***	R	R	S	R	S	S	R	S	S	R
	35±1	04±0.1	3.3±1.5	21±1	1.3±0.6	21.3±15	22.3±0.6	2.3±0.6	18.7±1.2	21±1	3.3±1.2

EHEC: Enterohaemorrhagic Escherichia coli; MRSA: methicillin resistant Staphylococcus aureus; C. pseudotuberculosis STS (Sheep isolate); C. pseudotuberculosis STG (goat isolate); R: resistant; S: susceptible. To be accurate, all isolates showed intermediately susceptible to specific antibiotic were categorized as resistant. Data were presented as mean ± SD of three independent experiments. **p<0.01 and ***p<0.001, indicate significance differences between inhibitory zones in millimeter of EOs and other antibiotics used in each bacterial isolate individually.

species; the oil was effective against the tested bacterial isolates except *Pseudomonas aeruginosa* and *Corynebacterium pseudotuberculosis* isolates from sheep and goats. Whereas, mint EO was powerful antibacterial active against *Salmonella newport, Staphylococcus aureus,* (MRSA) *Staphylococcus aureus, K. pneumonia* and *Mannheimia haemolytica*. Thyme, lavender, and mint essential oils were tested for their antifungal properties against fungal isolates. Results (Table 2) showed that all EOs had significant antifungal activity against the tested dermatophytes, with full inhibition observed. The fungal isolates were much more sensitive to Ketoconazole (KT) than other antifungal; significant difference in the inhibitory zones was found between Ketoconazole and other antifungal except Fluconazole against *Trichphyton mentagrophytes*. Nistatin (NS) was less active against all the fungi, with zero zones.

The broth dilution method was used to determine MIC, MBC and MFC concentrations of the examined EOs. The results are shown in Table 3 and 4. The studied bacterial and fungal isolates were more susceptible to the antimicrobial activity of thyme EO as compared to mint and lavender EOs. *E. coli, S. newport, S. aureus,* MRSA *S. aureus, C. pseudotuberculosis* STS and *C. pseudotuberculosis* STG were the most susceptible, with MBC values 0.9% indicating a strong antimicrobial activity of thyme EO. *P. aeroginosa* was found to be sensitive to thyme EO with MBC values 0.15%. Lavender and mint EOs were also found to be effective against *S. aureus,* MRSA *S. aureus* and *E. coli* with MBC values 0.9% and S. *newport* with MBC values 0.15%. The tested EOs displayed strong antimicrobial activity against the tested fungal isolates, with MFC values of 9µl/ml for thyme and 15µl/ml for both mint and lavender EOs.

DISCUSSION

The results of antibiotic susceptibility tests showed differences in the bacterial sensitivities to the studied antibiotics, where all of the bacterial isolates were sensitive to Ciprofloxacin. Studies reported that various Gram-positive and Gram-negative bacteria can be treated with ciprofloxacin, which is particularly effective against Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa.* By inhibiting DNA gyrase's A subunit and exerting additional influence on the components of cell walls, Ciprofloxacin prevents DNA replication (Shariati *et al.*, 2022). Imipenem was also found to be effective against the isolates used, except EHEC *E. coli*. This finding is in line with that reported by Iweriebor *et al*. (2022) who isolated imipenem-associated multidrug-resistant *E. coli* isolates from pork, and with that reported in antibiotic-resistant *E. coli* isolates from goat farms by Pomwised *et al.* (2023). Whereas, our results are in contrast to those found in *E. coli* isolated from various clinical sources from humans in Duhok city, Iraq (Naqid *et al.*, 2020). This is most likely due to the variations in the *E. coli* strains' sources that were tested in the two studies.

This study also found that most of the isolates were resistant to ceftriaxone, doxycycline, gentamicin, trimethoprim-sulfamethoxazole, and erythromycin. Antibiotic-resistant bacteria may emerge in areas of Duhok Province, Iraq, where the use of antibiotics in livestock is

Table 2: Antifungal resistant profile of the used dermatophytic isolates in this study compared to antimicrobial activities of EOs, Thyme, Lavender and Mint.

Antifungal	Fungal isolates	
	Microsporun canis	*Trichphyton mentagrophytes*
	Inhibition Zone diameter millimeter (mm)	
Ketoconazole (KT) 10 µg	32±9.8	36.5±2.12
Itraconazole (IT) 10 µg	21±5.6	15±3.5*
Miconazole (MC) 10 µg	16±1.4*	12.5±3.5**
Amphotericin (AP) 100 µg	13.5±2.1*	2***
Nistatin (NS) 50 µg	0	0
Fluconazole (FL) 25 µg	0	31.5±4.9
	Essential oils	
Thyme 10µl	Complete inhibition	Complete inhibition
Lavender 10 µl	Complete inhibition	Complete inhibition
Mint10 µl	Complete inhibition	Complete inhibition

Values shown as mean ± SD of three independent experiments. P-values indicate the significant differences between the inhibitory zones induced by Ketoconazole and other tested antifungals on *M. canis* and *Trichphyton mentagrophytes*. * = p<0.05, ** = p<0.01 and ***= p<0.001.

Table 3: MBC (µl/ml) values of the EOs against the bacterial isolates

Bacterial isolates	Thyme EO		Lavender EO		Mint EO	
	MIC (µl/ml)	MBC (µl/ml)	MIC (µl/ml)	MBC (µl/ml)	MIC (µl/ml)	MBC (µl/ml)
E.coli (EHEC)	6	9	9	9	9	9
S. newport	6	9	9	15	12	15
S. aureus	6	6	9	9	9	9
P. aeroginosa	12	15	R 21	R 21	R 21	R 21
Staphylococcus aureus (MRSA)	6	9	6	9	6	9
K. pneumoniae	6	9	6	15	6	12
P. multocida	9	12	12	15	R 21	R 21
M. haemolytica	9	12	12	15	12	15
C. pseudotuberculosis STS (ON142642)	6	9	R 21	R 21	R 21	R 21
C. pseudotuberculosis STG (ON142653)	6	9	R 21	R 21	R 21	R 21

Table 4: MFC (µl/ml) values of the EOs against the fungal isolates

Fungal isolates	Thyme EO		Lavender EO		Mint EO	
	MIC (µl/ml)	MBC (µl/ml)	MIC (µl/ml)	MBC (µl/ml)	MIC (µl/ml)	MBC (µl/ml)
M. canis	6	9	9	9	9	12
T. mentagrophytes	9	9	9	15	12	15

unrestricted, random, and applied by the owners of the animals. This disturbing discovery necessitates quick action to stop the potentially dangerous spread of multidrug-resistant bacteria among the local livestock population and subsequently, the local population.

Consistent with Singh *et al.* (2019), our results found that the fungal isolates were much more sensitive to ketoconazole (KT) than other antifungals. Whereas fluconazole (FL) was found to be effective against *Trichphyton mentagrophytes*, which is in line with that reported by Lalvand *et al.* (2021), but ineffective against *Microsporun canis*, which is in accordance with Singh *et al.* (2021). Nistatin (NS) was less active against all the fungi, with zero zones. Topical nystatin application in treating dermatophyte infections is restricted due to its relatively low minimal inhibitory concentration and minimal fungicidal concentration when compared to other topical antifungals (Muddasani and Rivin, 2023).

This study also showed that thyme EO exhibited potent antibacterial activity against every tested strain of bacteria; *E. coli*, *S. newport*, *S. aureus*, MRSA *S. aureus*, *C. pseudotuberculosis* STS and *C. pseudotuberculosis* STG were the most susceptible, with MBC values 0.9 % in broth dilution assays. This is most likely because the oil contains over 40% of phenolic compounds with antibacterial qualities, like carvacrol and thymol (Thosar *et al.*, 2013). The data are in line with those reported earlier by Abdelhamed *et al.* (2022). It has been found that both thymol and carvacrol cause disruption of the bacterial plasma membrane (Trombetta *et al*., 2005).

The antibacterial properties of the other two essential oils, lavender and mint, varied from isolate to isolate when tested. Lavender EO was not able to stop *P. aeruginosa* or *C. pseudotuberculosis* from growing in broth dilution assays even at 21µl/ml. These findings are in line with those reported by Tarek *et al.* (2014) and Adaszyńska-Skwirzyńska *et al.* (2023) who found that *P. aeruginosa* was resistant to lavender oil. This suggests that *P. aeruginosa* has evolved a variety of cellular defense mechanisms in response to unfavorable environmental circumstances, which could account for the bacteria's reported reduced susceptibility to lavender essential oils. Regretfully, there was no prior publication to compare our findings with regarding the susceptibility of *Corynebacterium pseudotuberculosis* to lavender EO; nevertheless, Awadalla *et al.* (2022) discovered that *Corynebacterium stationis* was resistant to lavender EO. One explanation might be that *Corynebacterium pseudotuberculosis* contains a thick coating of peptidoglycan, which could prevent many of the EOs from damaging membranes. The distinctive cell wall architecture of the genus Corynebacterium is defined by the presence of complex lipids and peptidoglycans, which make up 60% of the cell wall structure (Rebouças *et al.*, 2020).

Regarding the mint EO, the data found that, in addition to *P. aeruginosa* and *C. pseudotuberculosis, E. coli* and *P. multocida* were also resistant. The finding regarding *P. aeruginosa* is in line with Tarek *et al.* (2014). As mentioned above, it was hard to find publications to compare our findings with regarding the susceptibility of *Corynebacterium pseudotuberculosis* to EOs; however, a study tested the efficacy of terpinolene as a monoterpene found in EOs from several genera of plants, including Mentha, on *Corynebacterium pseudotuberculosis* and found it ineffective in inhibiting bacterial growth even at high concentrations (Paluso, 2019). Similarly, Van *et al.* (2022) found that peppermint EOs had no antibacterial activity on *E. coli* strains. Differently, Thompson *et al.* (2013) found good activity of mint EO against *E. coli* strain DH5α and Karagözlü *et al.* (2011) against *E. coli* O157:H7. The difference is probably due to the differences in the bacterial strains used in these studies; alternately, the variations may arise from variations in the composition of the tested oils, which may be explained by the variety of mint plant species used; the age, location, and processing conditions of the plant can affect the chemical composition

of peppermint essential oil (Beigi *et al.*, 2018) and the antibacterial activity of an EO may differ depending on its composition (Arámbula *et al.*, 2019). Our data found that mint EO was not able to completely inhibit the growth of *P. multocida*, which was in line to that reported by (Bismarck *et al*., 2022) who reported the inhibitory zone induced by peppermint EO against the bacteria at 13.5 mm by the agar disc diffusion method using 10 µl, whereas, our data is in contrast to that reported by Van *et al.* (2022) who found that the bacteria was strongly inhibited by peppermint oil in the broth dilution assays. This could be due to the high concentration of ≥219 mg/ml of mint EO used by the author compared to that we used in this study, which was 21 µl/ml.

In the study, we also investigated the antimicrobial activity of thyme, lavender, and mint EOs against animal clinical fungal isolates. The data found that the tested dermatophytes were strongly inhibited by the tested EOs in both the agar disc diffusion method and the broth dilution assay. There was a noticeable fungicidal impact of the used EOs on the tested dermatophytes, as the MIC for the majority of EOs was equal to 9µl/ml. The study's findings of inhibition for thyme EO were in line with previous studies that have demonstrated that thyme essential oils inhibited a variety of fungi, including dermatophytes (Parrish *et al.*, 2020). The mechanism of the essential oil-mediated inhibition was proposed to be the binding of thymol to ergosterol, which modifies membrane permeability and suppresses hyphal growth and conidia formation (Kowalczyk *et al*., 2020). Furthermore, it has been discovered that the phenolic monoterpene carvacrol depolarizes eukaryotic cells and disrupts the cell cycle and plasma membrane (Dai *et al.*, 2016). Likewise, our findings are consistent with those reported earlier by Ibrahim and Abd El-Salam (2015) who found a potent antidermatophyte by *Mentha piperita* against the tested *Microsporum canis*, *Epidermophyton floccosum*, *Trichophyton rubrum* and *Trichophyton mentagrophytes by* both the agar disc diffusion method and the broth dilution assay. Further, our data are in agreement with that reported by Zuzarte *et al.* (2011) who showed potent antifungal activities of *Lavandula viridis* against the tested dermatophytes and *Cryptococcus neoformans*, suggesting that this was due to α-pinene as an active compound, particularly against dermatophyte strains; α-pinene causes cell membrane disruption through actively binding to ergosterol in the cellular membrane.

Conclusion: The study found that bacteria and fungi have become resistant to various drugs, including popular antibiotics, indicating a potential threat to livestock populations and local communities and emphasizing the need for immediate action to prevent the spread of multidrug-resistant bacteria. Thyme essential oil demonstrated exceptional antibacterial and antifungal properties and effectively inhibited the growth of all tested bacteria and fungi strains.

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