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

Nawzat Abozaid Issa

Surgery and Internal Medicine department, College of Veterinary Medicine, University of Duhok, Kurdistan region, Iraq
*Corresponding author: nawzat.issa@uod.ac

**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.

**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, carvophyllene, terpinolene, β-myrcene, and borneol, cineol, linalool, menthone, B-cymene, pinene, and triterpenic acid (Dong et al., 2023; Thosar et al., 2013). 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 monoterpenoids and sesquiterpenoids; 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. (Kozuharova et al., 2023; Pokajewicz et al., 2021). 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, menthol 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 Itraconazole, Amphotericin, Fluconazole, Ketoconazole, Nystatin, and Miconazole. The isolates were classified as susceptible or resistant, with resistant isolates being intermediate 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 24-48 hours, 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-pioints). The isolates were classified as susceptible or resistant (it was decided to classify as resistant isolates those that were intermediate 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 (Jarjess and Issa, 2022) and Corynebacterium pseudotuberculosis (Sheep isolate (ON142642) and goat isolate (ON142653)) were isolated from clinically infected sheep and goats with caseous lymphadenitis (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 10⁶ 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 5x10⁶ 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 37°C for bacteria and four weeks for fungi at 30°C 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
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 *Trichophyton 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. aeruginosa* 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 9 µl/ml for thyme and 15 µl/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 wall, 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 Iwerehbor 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 Pimwised 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 ceftiraxone, 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 1: Antibiotic resistant profile of the used bacterial isolates in this study compared to antimicrobials activities of EOs, Thyme, Lavender and Mint.

<table>
<thead>
<tr>
<th>Bacterial Isolates</th>
<th>Thyme 10 µl</th>
<th>Mint 10 µl</th>
<th>Lavender 10 µl</th>
<th>Impen 10 µg</th>
<th>Trimethoprim-sulfamethoxazole 75 µg</th>
<th>Erythromycin 10 µg</th>
<th>Ciprofloxacin 10 µg</th>
<th>Gentamicin 10 µg</th>
<th>Norfloxacin 30 line</th>
<th>Doxycycline 10 ne 30 µg</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli (EHEC)</td>
<td>S*** R</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>S. newport</td>
<td>S*** S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>S. aureus</td>
<td>S*** S***</td>
<td>S*** S***</td>
<td>S*** S***</td>
<td>S*** S***</td>
<td>S*** S***</td>
<td>S*** S***</td>
<td>S*** S***</td>
<td>S*** S***</td>
<td>S*** S***</td>
<td>S*** S***</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>S R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>S*** S</td>
<td>S S</td>
<td>S*** R</td>
<td>S*** R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>aureus (MRSA)</td>
<td>35.7±0.6</td>
<td>16.7±1</td>
<td>22.3±3.2</td>
<td>2.3±0.6</td>
<td>11.7±0.6</td>
<td>2.1±7</td>
<td>8.7±3.1</td>
<td>22.3±6.7</td>
<td>5±1.4</td>
<td>5±1.4</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>S S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>P. multocida</td>
<td>27±2.6</td>
<td>20</td>
<td>21.7±2.7</td>
<td>7.7±0.6</td>
<td>2.7±0.6</td>
<td>20.3±8.3</td>
<td>5.5±0.7</td>
<td>22.7±6.4</td>
<td>14.3±1.2</td>
<td></td>
</tr>
<tr>
<td>M. haemolytica</td>
<td>26.3±7.6</td>
<td>16.3±2</td>
<td>22±2.6</td>
<td>8.3±0.6</td>
<td>5.3±0.6</td>
<td>23.7±5.5</td>
<td>6±2.6</td>
<td>3.3±1.2</td>
<td>6±1</td>
<td></td>
</tr>
<tr>
<td>C. pseudotuberculosis</td>
<td>S*** S</td>
<td>S</td>
<td>S*** R</td>
<td>S*** R</td>
<td>R</td>
<td>S</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
</tr>
<tr>
<td>sis STS</td>
<td>31.3±1.2</td>
<td>4.3±1.5</td>
<td>19±1</td>
<td>2.7±0.6</td>
<td>22.3±0.6</td>
<td>25.7±0.6</td>
<td>2.7±0.6</td>
<td>18.7±0.6</td>
<td>10.7±2.3</td>
<td>23.6±0.6</td>
</tr>
<tr>
<td>C. pseudotuberculosis</td>
<td>35±1.0</td>
<td>04±0.1</td>
<td>3.3±1.5</td>
<td>21±1</td>
<td>1.3±0.6</td>
<td>21.3±15</td>
<td>22.3±0.6</td>
<td>6.3±0.6</td>
<td>18.7±2.1</td>
<td>3.3±1.2</td>
</tr>
</tbody>
</table>

EHEC: Enterohaemorrhagie 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 immediately susceptible to specific antibiotic were categorized as resistant. Data were presented as mean ± SD of three in 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.
Antifungal resistant profile of the used dermatophytic isolates in this study compared to antimicrobial activities of EOs, Thyme, Lavender and Mint.

<table>
<thead>
<tr>
<th>Antifungal</th>
<th>Microsporon canis</th>
<th>Trichophyton mentagrophytes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ketoconazole (KT) 10 µg</td>
<td>32±9.8</td>
<td>36±5.2±12</td>
</tr>
<tr>
<td>Itraconazole (IT) 10 µg</td>
<td>2±1.5</td>
<td>15±3.5*</td>
</tr>
<tr>
<td>Micronazole (MC) 10 µg</td>
<td>13±1.4*</td>
<td>12.5±3.5**</td>
</tr>
<tr>
<td>Amphotericin (AP) 100 µg</td>
<td>13.5±2.1*</td>
<td>2***</td>
</tr>
<tr>
<td>Nistatin (NS) 50 µg</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Fluconazole (FL) 25 µg</td>
<td>0</td>
<td>31.5±4.9</td>
</tr>
<tr>
<td>Esential oils</td>
<td>Complete inhibition</td>
<td>Complete inhibition</td>
</tr>
<tr>
<td>Thyme 10µl</td>
<td>Complete inhibition</td>
<td>Complete inhibition</td>
</tr>
<tr>
<td>Lavender 10 µl</td>
<td>Complete inhibition</td>
<td>Complete inhibition</td>
</tr>
<tr>
<td>Mint 10 µl</td>
<td>Complete inhibition</td>
<td>Complete inhibition</td>
</tr>
</tbody>
</table>

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 Trichophyton mentagrophytes, which is in line with that reported by Lalvand et al. (2021), but ineffective against Microsporon 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 (Trombeta 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 2µl/ml. These findings are in line with those reported by Tarek et al. (2014) and Adaszynska-Skwarzynska 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 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 Karagözli 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 monoterpenic 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.

---

**REFERENCES**


