Antibacterial Activity of Herbal Essential Oils against Gram-positive and Gram-negative Bacteria with a Potential for Multidrug Resistance

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Abstract

Background: In recent years, there has been an extensive and great interest in researching and developing alternative antibacterial agents from various sources to combat bacterial resistance. Therefore, greater attention has been paid to the screening of antibacterial activity. **Aims**: This study evaluated different types of herbal essential oil to inhibit the growth of selected human pathogenic bacteria with potential multidrug resistance properties. **Methodology**: The herbal essential oils used include thyme oil, tea tree oil, bergamot oil and lavender oil against *Staphylococcus aureus, Escherichia coli and Bacillus subtilis*. Antibacterial screening of all the essential oils was done by disk diffusion method. **Results**: Herbal essential oils have exhibited a broad spectrum of antibacterial activities against the selected microbes: *B. subtilis, S. aureus* and *E. coli.* Thyme oil has the most potential antibacterial properties for *B. subtilis*(40 mm), followed by *S. aureus*(35 mm) and *E. coli*(35 mm). Both bergamot and lavender oils showed higher antibacterial effects for *E. coli* and *B. subtilis* but have lower efficacy with *S. aureus* than tea tree oil. **Conclusion**: The herbal essential oils used in this study can serve as a source of alternative antibacterial agents and may play an important role in the discovery of new drugs for the treatment of a wide range of pathogenic bacteria in the future.

Keywords: Antibacterial activity; *Bacillus subtilis; Staphylococcus aureus; Escherichia coli*; essential oils; herbs; thyme; tea tree; lavender; bergamot

Significance | This research showed that essential oils derived from medicinal plants are antibiotic-resistant by exhibiting strong antibacterial activity against multidrug-resistant pathogens, suggesting potential alternative options against antibiotic resistance.

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1. Introduction

The global health community is increasingly concerned about the spread and emergence of antibiotic resistance, as well as the evolution of new strains of disease-causing agents. Recent reports have indicated that many healthcare-associated, antimicrobial-resistant bacteria are not only multidrug-resistant pathogens but also broadly drug-resistant and pandrug-resistant bacteria (Singh & Katoch, 2020). Medicinal plants, known for their therapeutic properties and beneficial pharmacological effects on the human body, are recognized as alternative sources of drugs that could potentially be effective in treating these problematic bacterial infections (Salem *et al.*, 2023, Al-Haj *et al.*, 2019, Boadu & Asase, 2017).

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a Essential oils, which are volatile terpene hydrocarbons, and their oxygenated derivatives are secondary metabolites, are known for their concentrated natural pharmacologically active ingredients (Boadu & Asase, 2017). They have been used in folk medicine, traditional and alternative medicine for a long time. The antibacterial activity of essential oils has been recognized for many years, and their preparations find wide application as naturally occurring antibacterial agents in pharmacology, medical and clinical microbiology (Chukwunwike *et al.*, 2019, Lim *et al.*, 2022).

Antibacterial resistance is a major global public health concern. Bacteria produce the resistance mechanisms with biofilm and different ESKAPE mechanisms (Mariam et al. 2024, Hiba et al. 2024). It is a natural consequence of pathogens adapting to exposure to antibacterial agents used in medicine, food, and disinfectants in households (Van Dijk *et al.*, 2022; Li & Webster, 2018). Resistant infections increase treatment costs, disease spread, and illness duration (Dadgostar, 2019). To address this challenge, there is a need to find novel antibacterial agents with new modes of action. Many plant species have been reported to act against various bacteria in vitro, and many medicinal plants produce secondary metabolites (phytochemicals) capable of inhibiting the growth of a wide range of microorganisms (Nwonu *et al.*, 2019; Rashid *et al.*, 2017). Recently developed nanoparticle formualtion also shows promise for the treatment of antibiotic resistance (Muntaha and Dhafar, 2024). Therefore, the present study aimed to examine the antibacterial activity of selected essential oils against human pathogenic bacteria.

2. Materials and methods

Source of essential oils

Light thyme oil (*Thymus vulgaris*), bergamot oil (*Citrus bergamia*), lavender oil (*Lavandula angustifolia*) and tea tree oil (*Melaleuca alternifolia*) were selected based on literature survey and their use in traditional medicine. Quality of the oils was ascertained to be more than 98% pure. The essential oils were provided by the private limited company *Tanamera* Tropical Spa*, Malaysia.*

Preparation of essential oils standard concentrations

The dilution of the essential oils was done using dimethyl sulfoxide (DMSO) (*Sigma-Aldrich, Germany)* which was selected as a solvent because it does not have any antibacterial activity unlike other solvent (Tween 80). DMSO is also considered to be less toxic than solvent containing halogen or benzene and is volatile enough to be removed rapidly by drying at room temperature (Almola, *et al.*, 2016). The essential oils obtained were dried over anhydrous sodium sulphate and after filtration, Gas Chromatography (GC) analyses used to prepare pure oils were performed with a Varian CP-3800 gas chromatograph equipped with a DB-5 capillary column (30 m x 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector.

Source of selected human pathogen bacteria

Three selected human pathogenic bacteria *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* were obtained from microbiology laboratory of Lincoln University College. A series of morphological test by Gram staining and microscopic examination, physiological test by culture on Nutrient Agar (NA) and MacConkey Agar (MAC) (HiMedia, India), and conventional biochemical tests were performed to identify the selected microorganisms (Mindy, *et al.*, 2003). The Gram staining method was carried out to characterize the isolated bacteria under the microscopy and morphological characters were recorded. The characteristics such as color, elevation, pigmentation, shape, size, surface, margin, odor, etc., of the bacteria *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilis* on the NA and MAC media were recorded. The Biochemical test carried out by Analytical Profile Index (API staph, and API 20E test, bioMerieux, Inc., France) and results were recorded (Al-Haj, *et al.*, 2019).

Antibacterial susceptibility test by using Disk Diffusion Assay

Antibacterial susceptibility test was performed for all microbial isolates by modified Kirby Bauer disc diffusion method following the guideline by the Clinical and Laboratory Standards Institute (2020). Multidrug resistant (MDR) isolates were defined as those isolates that are resistant to four type of test antibiotics (Gentamicin CN, Tetracycline TE, Amoxicillin AMP and Streptomycin S) (HiMedia, India) tested on Mueller Hinton Agar (MHA- Becton, Dickinson, France) plates with 60 mg/mL concentration of each essential oils were prepared in 10% in DMSO. The selected human pathogenic bacteria cell suspensions were adjusted to 0.5 McFarland turbidity standards to prepare 1×10^8 bacterial/mL inoculum. Each bacterial suspension was inoculated on Mueller-Hinton agar plates, then allowed to dry for 5 minutes. The sterile filter paper discs (Whatman No. 1, diameter = 5 mm) were soaked in 10 μL of each essential oil. The extract-soaked filter paper discs were then placed on the inoculated Mueller-Hinton agar plates, 10% DMSO-soaked filter paper disk was used as the negative control. Plates were incubated for 18 h at 35 ± 2 °C. After incubation, the zones of inhibition were recorded as the diameter of the growth-free zones measured in mm using a sterile cork-borer (5 mm).

Minimum Inhibitory Concentration (MIC)

Essential oils that gave a positive result for the disk diffusion assay were used to determine MIC using the microplate dilution method. Serial 2-fold dilutions of the essential oils were prepared in the 10% DMSO in each well. Inoculum of organism 5×10^5 CFU/mL was prepared in Mueller-Hinton broth, and the turbidity was adjusted to approximately 0.5 McFarland turbidity standard to prepare 1×10^8 bacterial/ml. 500 μL of the suspensions were spread over the plates that contain the agar media using a sterile cotton swab in order to get a uniform microbial growth on both control and test

Table 1. Antibiotics susceptibility against selected pathogenic bacteria. (S: Sensitive, I: Intermediate, R: Resistant, according to CLSI guideline 2020).

Antibiotic susceptibility	Pathogenic bacteria zone of inhibition (mm)		
	S.aureus	***E.coli***	***B. subtilis***
Gentamicin (CN)	21 (S)	21 (S)	14 (I)
Tetracycline (TE)	13 (I)	11 (R)	10 (R)
Amoxicillin (AMP)	0	0	0
Streptomycin (S)	19 (S)	19 (S)	11 (R)
DMSO	0	0	0

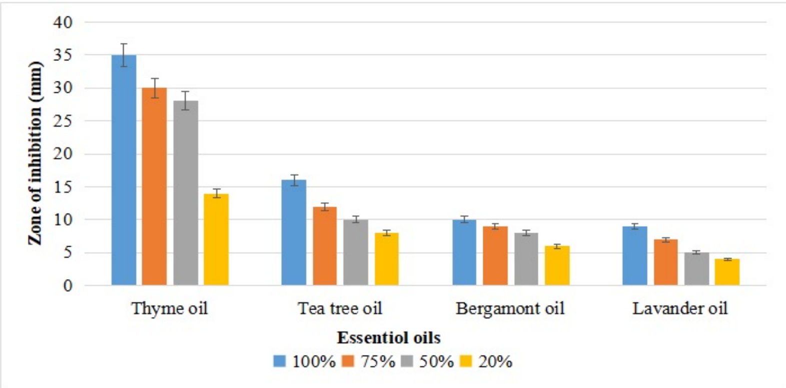

Figure 1. Antibacterial activity of different concentrations of essential oils against *Staphylococcus aureus*

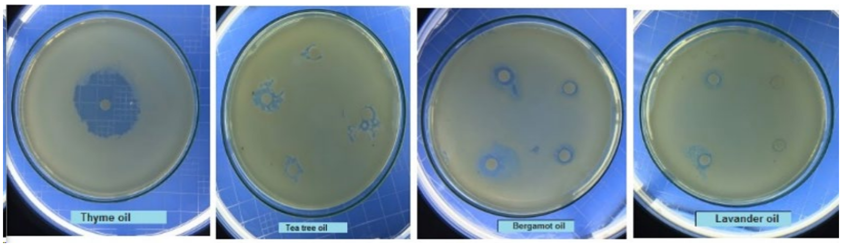

Figure 2. Antibacterial activity of different concentrations of essential oils against *Staphylococcus aureus.*

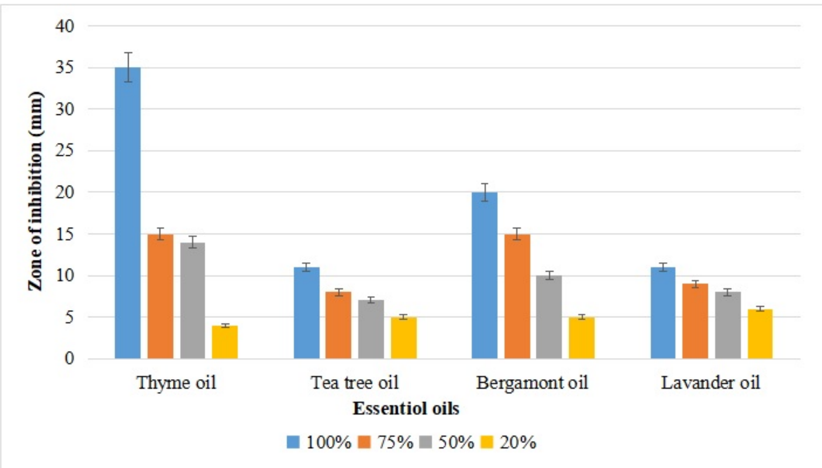

Figure 3. Antibacterial activity of different concentrations of essential oils against *E. coli.*

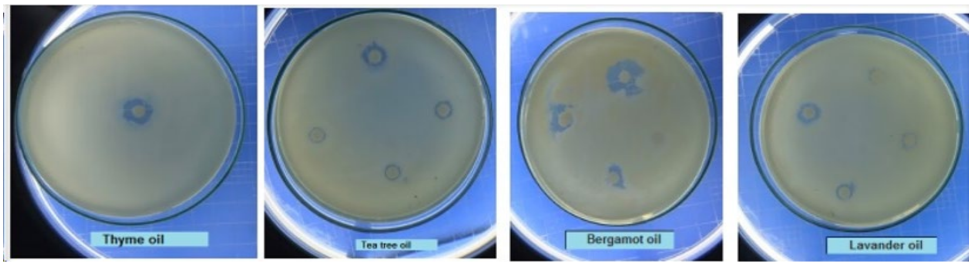

Figure 4. Antibacterial activity of different concentrations of essential oils against *E. coli.*

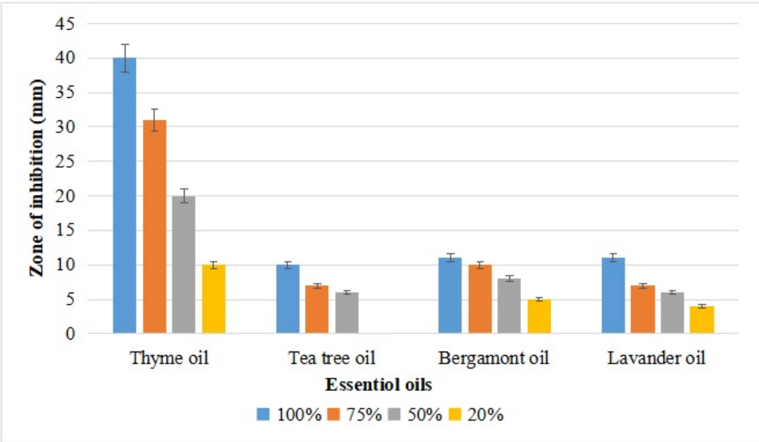

Figure 5. Antibacterial activity of different concentrations of essential oils against *B. subtilis.*

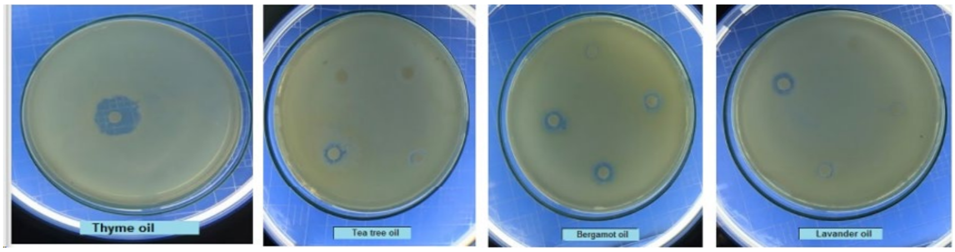

Figure 6. Antibacterial activity of different concentrations of essential oils against *B.subtilis.*

plates. An empty sterilized disc (5 mm diameter, Whatman no. 3, Germany) were impregnated with 50 µL of different concentrations of each essential oil (100%, 75%, 50% and 20%) and placed on the inoculated agar surface. Paper disc moistened with DMSO wasplaced on the seeded Petri plate as a vehicle control, to prove that there is no antimicrobial effect for the solvent. The Petri-plates after that were kept for 30 min at 30°C, to allow the diffusion of the oils. After overnight incubation, plates were observed for the formation of a clear zone around the disc which corresponds to the antimicrobial activity of tested compounds. The zone of inhibition was observed and measured in mm. All experiments were performed in duplicate, and mean value was determined.

Data analysis method

All experiments were independently repeated three times. Data were analyzed and calculated using EXCEL (Microsoft Office 2016, Microsoft, USA).

3. Results

Antibiotics susceptibility of selected pathogenic bacteria

Table 1 shows the antibiotics susceptibility of *S. aureus, E. coli* and *B. subtilis*. For Gentamicin, the highest inhibition zone is 21 mm against *S. aureus, E. coli* and moderate against *B. subtilis,* followed by Streptomycin 19 mm against *S. aureus, E. coli* and 11 mm against *B. subtilis*. Tetracycline showed moderate activity against all microorganism but there are resistance activity for Amoxicillin (AMP). The negative control (DMSO) showed no effect against selected pathogenic microorganism.

Evaluation of essential oils bioactivity against selected pathogenic bacteria

The antibacterial activities for the different concentrations of the essential oils against *S. aureus* are presented in Figure 1 and 2. Thyme oil revealed the strongest effect with 35 mm (sensitive) even with lowest concentration against *S. aureus* followed with effect of tea tree oil. In contrast, thyme oil (20%) revealed the minimum concentration zone of inhibition is 14 mm (intermediate) and tea tree oil (20%) with 8 mm (resistant) zone of inhibition in the beginning with high rate of antibacterial activity. The beginning high rate of minimum concentration (20%) of the essential oils interact with cell membrane proteins at different sites of the cell and affect a variety of cellular functions.

The antibacterial activities for the different concentrations of the essential oils against *E. coli* are presented in Figure 3 and 4. Figure 3 shows the highest antibacterial activity of thyme oil and bergamot oils against *E. coli* with a zone of inhibition of 35 and 20 mm (sensitive) respectively. Figure 4 reveals that tea tree and lavender oils have similar activity with a zone of inhibition of 11 mm (resistant) respectively, according to the guideline by the Clinical and Laboratory Standards Institute (2020).

Figure 5 and 6 shows the zone of inhibition against *B. subtilis* using the essential oils thyme, bergamot, lavender and tea tree with a zone of inhibition of 40 mm (sensitive), 11 mm (resistant) , 11 mm (resistant) and 10 mm (resistant) respectively, according to the guideline by the Clinical and Laboratory Standards Institute (2020).

4. Discussion

Numerous naturally occurring compounds found in plants, herbs, and spices have demonstrated antibacterial properties and potential as sources of antibacterial agents against pathogens (Chouhan *et al.*, 2017, Swamy *et al.*, 2016). Microbial infectious diseases are significant contributors to global morbidity and mortality, highlighting the need for the development of new antibacterial agents. The antibiotics tested (Tetracycline TC, Gentamicin CN, Streptomycin S, and Amoxicillin AMP) exhibited antibacterial effects against the tested bacteria. Gentamicin was the most effective against all bacteria, while Amoxicillin showed the lowest antibacterial activity (Salem, *et al*., 2023).

Thyme oil demonstrated activity against all tested bacterial strains, with varying effectiveness depending on concentration and bacterial type. The concentrations of thyme oil generally exhibited higher activity than the selected standard antibiotics, as evidenced by their zones of inhibition. This finding aligns with previous reports by Pancu *et al.* (2022) on the antibacterial activity of essential oils, including thyme, against various pathogens.

Tea tree essential oil, known for its antibacterial properties attributed to small terpenoids and phenolic compounds (such as thymol, carvacol, and eugenol), showed high antibacterial activity, particularly against S. aureus. This result is consistent with reports by Singh & Katoch (2020) regarding the antibacterial activity of 4-terpineol and α-terpineol, which are present in tea tree oil. A study conducted by Oliva *et al.* (2018) also showed that tea tree oil exhibited a potent bactericidal activity against methicillin-sensitive *S. aureus* (MSSA), *E. coli*, and clinical strains of methicillin-resistant *S. aureus* (MRSA), extended-spectrum beta lactamases producer carbapenem-sensitive *Klebsiella pneumoniae* (ESBL-CS-Kp), carbapenem-resistant *K. pneumoniae* (CR-Kp), *Acinetobacter baumannii* (CR-Ab), and *Pseudomonas aeruginosa* (CR-Pa). Tea tree oil in combination with each reference antimicrobial showed a high level of synergism at sub-inhibitory concentrations, especially with oxacillin (OXA) against MRSA (Oliva *et al.*, 2018).

In contrast, bergamot essential oil exhibited the lowest antibacterial activity. The complexity and variability of essential oils make it difficult to correlate their antibacterial activities with specific compounds. However, it is generally explained by their secondary metabolites, such as terpenes with aromatic rings and phenolic hydroxyl groups capable of forming hydrogen bonds with target enzymes. The lack of these compounds in bergamot oil could account for its lower antibacterial activity (Lim, *et al.*, 2022).

Lavender essential oil is well-documented for its in vitro antibacterial activity, although variations in research methods make specific references challenging (Kwiatkowski *et al.*, 2019, Hossain *et al.*, 2017). Our findings regarding lavender oil's antibacterial activity differed from those of previous studies, possibly due to variations in extraction methods. The tight closure of the wells in our impedimetric test prevented the essential oils from evaporating, ensuring consistent oil amounts throughout the test. Additionally, the presence of halitosis likely contributed to the oils' antibacterial effects in this system. Recent studies emphasize the role of water-soluble and vaporized components in assessing essential oils' antibacterial activity (Truong & Mudgil, 2023). The smaller probe volume in our study likely contributed to the essential oils' quicker antibacterial activity against microorganisms, making the system less inert (Singh & Katoch, 2020).

The implication of this study demonstrates the potential antibacterial properties of thyme oil, tea tree oil, bergamot oil and lavender oil against *S. aureus, E. coli and B. subtilis.* Thus, opportunities exist for fellow researchers to examine different herbal essential oils and they antipathogenic (antibacterial, antifungal, and antiviral) effects. Moreover, the ongoing incidence of emerging and re-emerging infectious diseases with the threat of impending pandemics (Najimudeen *et al.*, 2022, Chen *et al.*, 2022, Abdalqader *et al.*, 2020) highlights the necessity in exploring potential antipathogenic activities, not limited to antibacterial properties of essential oils. This study focused mainly on examining the antibacterial properties of herbal essential oils on some common bacteria that can be multidrug-resistant. Future work could now extend this work and explore other antipathogenic activities of essential oils especially against viruses, as well as other multidrug-resistance bacteria.

5. Conclusion

The essential oils used in this study exhibited a broad spectrum of antibacterial activities against both Gram-positive and Gram-negative bacteria with a potential for multidrug resistance. Thyme oil has the most potential antibacterial properties for all the selected bacteria: *B. subtilis*, *S. aureus* and *E. coli.* However, both bergamot and lavender oils revealed weaker antibacterial effects on *S. aureus* and showed higher antibacterial effects for *E. coli* and *B. subtilis* than tea tree oil. Thus, essential oils of medical and aromatic plant might be a prospective source of alternative antibacterial agents and may play an important role in the discovery of new drugs for the treatment of a wide range of pathogenic bacteria in the near future.

Author contribution

A.Y.A., A.A.A.A., M.F.B., H.W.J.C., M.N.A., H.A.A.A., I.I.A., M.A.M.A., J.I.S., A.S., R.A., E.S.A., B.H..S. conceptualized, wrote, reviewed, analyzed data, performed the experiment and edited the manuscript. All authors read and agreed to the published version of the manuscript.

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Competing financial interests

The authors have no conflict of interest.

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