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Evaluating the antibacterial properties of essential oils from Arugula, Thyme, Cinnamon, Mint, and Myrrh against pathogenic bacteria

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Five essential oils-arugula, thyme, cinnamon, mint, and myrrh-were purchased from a local market Riyadh, Saudi Arabia, and evaluated for their antibacterial properties against four significant human pathogens: *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*, and *Bacillus subtilis*. The antibacterial effectiveness of these essential oils was assessed using the disc diffusion method. The minimum inhibitory concentration (MIC) was determined through a twofold serial dilution technique. Chemical profiling of the selected essential oils was conducted using gas chromatography-mass spectrometry (GC-MS). The findings revealed that all essential oils exhibited varying degrees of antibacterial activity. Mint and myrrh oils, in particular, showed strong inhibitory effects across all tested pathogens. The MIC values for mint and myrrh oils were found to be between 3.125 and 12.5 µl/ml, while the other essential oils ranged from 25.0 to 100 µl/ml. In the chemical analysis of mint oil, eight primary compounds were identified: limonene, eucalyptol, menthone, menthol, alpha-terpineol, isomenthol, germacrene D, and germacrene D-4-ol, with menthol being the predominant component at 68.9%. Conversely, myrrh oil was found to contain eight chemical constituents: monegene, menthofuran, beta-elemene, caryophyllene, curzene, cadinene, germacrene B, and furanoeudesma-1,3-diene, with curzene as the leading compound at 45.9%. This research highlights the potent antibacterial properties of mint and myrrh essential oils as natural alternatives to conventional antibiotics, addressing the urgent issue of antibiotic resistance. Their broad-spectrum activity, surpassing ciprofloxacin against certain pathogens, and low MIC values demonstrate their high efficacy and potential as cost-effective antimicrobial agents.

Keywords: Essential oils, Antibiotic resistance, Pathogenic bacteria, Mint, Myrrh

INTRODUCTION

Pathogenic bacteria are responsible for numerous significant infections and diseases in humans. The genus *Staphylococcus* is a common human pathogen, causing a variety of infections, including skin infections, pneumonia, and other severe conditions. *Streptococcus* species are also known to cause a range of infections, such as skin infections, pharyngitis, and pneumonia. *Escherichia coli* is associated with enteric diseases, sepsis, and urinary tract infections. *Bacillus subtilis*, a ubiquitous bacterium, can survive in harsh environments and is linked to secondary infections. Additionally, both *E. coli* and *Staphylococcus aureus* are major foodborne pathogens[1]. The misuse of antibiotics is a significant factor contributing to the development of resistance in these pathogens. Examples of antibiotic-resistant bacteria include Methicillin-Resistant *S. aureus* (MRSA) and Enterobacteriaceae producing Extended Spectrum β-lactamases (ESBL)[2]. The rate at which bacteria are developing resistance far exceeds the rate of new antibiotic discovery, which is a serious concern[3]. As a result, there is an urgent need for alternative treatments to address these bacteria, reduce the emergence of resistant

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strains, and decrease reliance on antibiotics[4].

A significant portion of the global population relies on traditional or home remedies for primary healthcare. Moreover, concerns about the side effects of synthetic chemicals on human health have prompted many to turn towards natural products. Various parts of medicinal and aromatic plants, including their extracts and oils, are commonly used to treat a wide range of ailments[5]. Essential oils, which are volatile aromatic oils extracted from different plant parts, are known for their bioactive properties. These oils have been documented for their antimicrobial, insecticidal, and anticancer effects. In traditional medicine, essential oils are frequently used to treat pain, digestive issues, burns, and skin conditions. Additionally, many essential oils are incorporated into beauty products and are prized for their rich fragrances [6].

Essential oils have been traditionally used across cultures for a variety of purposes. These oils are primarily composed of terpene derivatives and oxygenated hydrocarbons, which contribute to their bioactive properties[7]. Various studies have screened essential oils for their antibacterial effects, demonstrating their usefulness in combating infections, including those caused by resistant bacteria[8]. Thus in the present study five essential oils available in the market of Saudi Arabia were evaluated for the antibacterial activity.

Materials and methods

Essential oils and bacterial strains

The essential oils of Arugula (*Eruca vesicaria)*, Thyme (*Thymus vulgaris*), Cinnamon (*Cinnamomum verum*), Mint (*Mentha arvensis*), and Myrrh (*Commiphora* sp.) were procured from local markets in Riyadh, Saudi Arabia, for the study. These oils were selected for their known medicinal properties, particularly their antimicrobial activities, and were evaluated for antibacterial potential in the subsequent experiments. In this study, four bacterial strains- *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25928, *Streptococcus pyogenes* ATCC 12384, and *Bacillus subtilis* ATCC 6633 -were employed to evaluate the antibacterial efficacy of essential oils. The disc diffusion method was utilized for assessing antibacterial activity. Bacterial cultures were prepared in nutrient broth by inoculating a small amount of each bacterial strain and incubating for 18 hours. A 10 µl inoculum (1×10^6 CFU/ml) was evenly spread onto Mueller-Hinton agar plates. After allowing the plates to rest for one hour, sterile filter paper discs saturated with 50 µl of essential oil were placed onto the agar surface. The plates were then incubated at 37°C for 24 hours. The antibacterial activity was determined by measuring the diameter (in mm) of the clear zone of inhibition (ZOI) surrounding the discs. Ciprofloxacin discs were used as a positive control. All experiments were performed in triplicate to ensure accuracy and reproducibility[9].

Determination of minimum inhibitory concentration (MIC)

To determine the minimum inhibitory concentration (MIC) of the essential oils, a double-fold dilution method was employed. In this procedure, 100 µl of each essential oil was serially diluted using sterile Eppendorf tubes. After dilution, 10 µl of bacterial culture (1×10^6 CFU/ml) as added to each tube, with nutrient broth serving as the diluent for the essential oils. For controls, sterile water was used as a negative control, and ciprofloxacin was used as a positive control. The tubes were then incubated at 37°C for 24 hours. After incubation, samples from each tube were streaked onto agar plates, which were again incubated at 37°C for another 24 hours. The lowest concentration of essential oil that completely inhibited bacterial growth, as observed by the absence of colonies on the agar plates, was recorded as the MIC value[10].

Gas chromatography-Mass spectrometry (GC-MS) analysis of essential oils

Gas chromatography-mass spectrometry (GC-MS) was utilized to identify the chemical constituents of the selected essential oils. The gas chromatograph used was a Perkin Elmer Clarus 500, equipped with a DB-5 capillary silica column (30 m x 0.25 mm; 0.25 µm film thickness). The initial oven temperature was set to 40°C for 1 minute, then increased to 240°C at a rate of 3°C/min. Helium was used as the carrier gas at a flow rate of 1.0 ml/min. The injector and detector temperatures were maintained at 250°C and 300°C, respectively, with a split ratio of 1:20. For GC-MS analysis, the same operational conditions were followed as for gas chromatography. Mass spectra were recorded at 70 eV, and the identification of chemical components was carried out using the NIST database for spectral interpretation[11].

Results

Five commercially available essential oils-arugula, thyme, cinnamon, mint, and myrrh-were tested for their antibacterial efficacy against four key human pathogenic bacteria*: E. coli, S. aureus, S. pyogenes,* and *B. subtilis*. The antibacterial activity was evaluated using the disc diffusion method, which demonstrated that all selected essential oils inhibited bacterial growth, though the degree of inhibition varied (Figure 1). Mint and myrrh oils exhibited the strongest inhibitory effects across all bacterial strains. The largest zone of inhibition (ZOI) was observed with mint oil against *B. subtilis* (28.0 mm), followed by *S. pyogenes* (26.33 mm), *S. aureus* (24.23 mm), and *E. coli* (21.5 mm). Myrrh oil produced similar results, with its highest ZOI recorded against *B. subtilis* (25.47 mm). Thyme oil showed its greatest effectiveness against *B. subtilis* (17.0 mm), and moderate activity against *E. coli* (15.0 mm), with lower

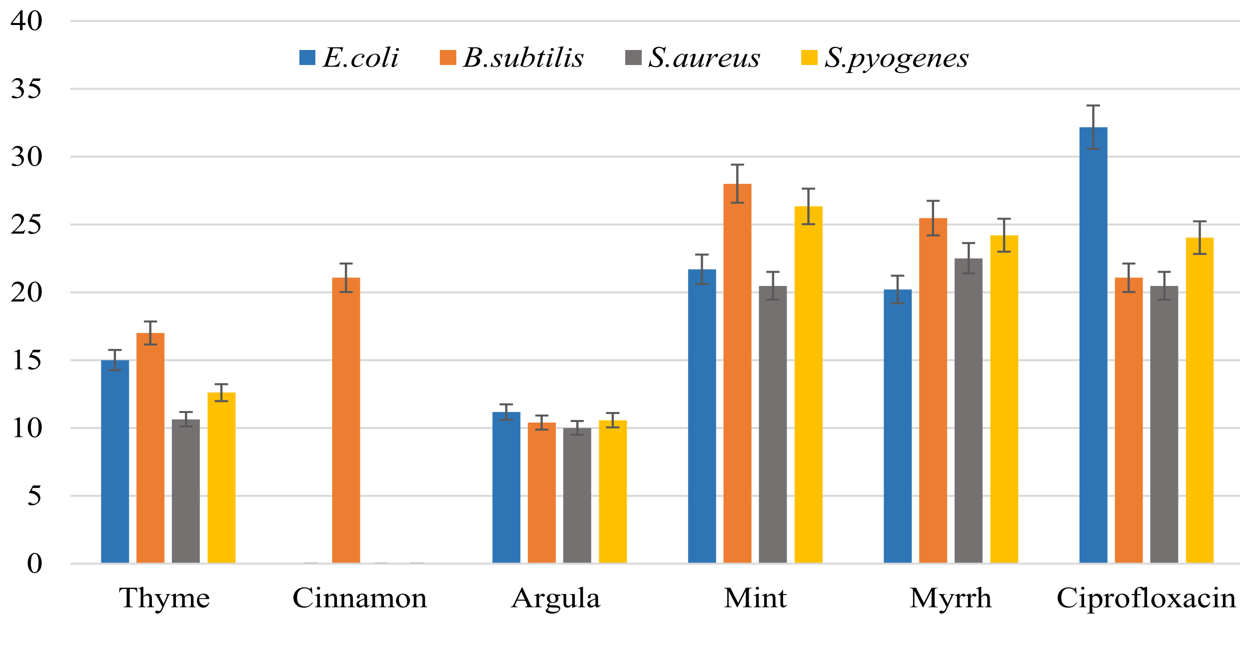

Figure 1. Antibacterial activity of essential oils of mint, arugula, cinnamon, myrrh and thyme against four pathogenic bacteria

efficacy against *S. aureus* (10.0 mm) and *S. pyogenes* (12.0 mm). Arugula oil demonstrated only weak antibacterial action against all tested pathogens, while cinnamon oil was solely effective against *B. subtilis* (12.5 mm). Notably, mint and myrrh oils outperformed the standard antibiotic ciprofloxacin in inhibiting the growth of *B. subtilis*, *S. aureus*, and *S. pyogenes*. Furthermore, these oils also displayed significant antibacterial activity even at lower concentrations, highlighting their potential as effective natural antimicrobial agents.

MIC of mint and myrrh oils ranged from 3.125 to 12.5 µl/ml, whereas the MIC for the other essential oils tested against the bacterial strains was between 25.0 and 100 µl/ml (Table 1). The lowest MIC, 3.125 µl/ml, was observed for mint oil against *B. subtilis* and *S. pyogenes*, and for myrrh oil against *B. subtilis* (Table 1). Due to their significant potential to inhibit bacterial growth, the chemical compositions of both mint and myrrh oils were analyzed.

The chemical analysis by GC-MS of mint oil revealed the presence of eight key compounds: limonene, eucalyptol, menthone, menthol, alpha-terpineol, isomenthol, germacrene D, and germacrene D-4-ol. Among these, menthol was the predominant component, making up 68.9% of the essential oil's composition (Table 2). On the other hand, the analysis of myrrh oil identified eight major constituents: monoterpene, menthofuran, beta-elemene, caryophyllene, curzerene, cadinene, germacrene B, and furanoeudesma-1,3-diene. Curzerene was the most abundant component, accounting for 45.9% of the essential oil (Table 3).

Discussion

Essential oils have been historically utilized for diverse medicinal and therapeutic applications worldwide[12]. Extensive research has been conducted on the antimicrobial potential of essential oils, particularly in relation to their ability to combat bacterial infections[6]. Numerous studies have evaluated the antibacterial properties of these oils, highlighting their potential as natural agents for treating infections, including those caused by antibiotic-resistant bacteria[12]. In this study, five commercially available essential oils were evaluated for the antibacterial potential against four human pathogenic bacteria. The essential oils were selected on the basis of their medicinal values and popularity among the local population. Myrrh (Frankincense) is known incense used in religious ceremonies also it is used in cosmetics, pharmaceutical products. There are reports on antiasmatic, antitronbotic, antitumor and antimicrobial potential of myrrh[13]. Thyme is a common culinary herb, also used for flavouring as well as has used in traditional medicine[14]. Cinnamon has important place in cooking, it is used for flavouring sweet and savoury dishes, and its oil has showed antibacterial properties[15]. Similarly, oil of arugula is used in food preparations besides it has been explored for its antibacterial activity[16]. Mint oil is well recognized for cosmetic, culinary, perfumery and therapeutic roles[12].

Table 1. The minimum inhibitory concentration (MIC) of essential oils of mint, arugula, cinnamon, myrrh and thyme against four pathogenic

Bacterial strain	Essential oil				
	Thyme	Cinnamon	Arugula	Mint	Myrrh
	MIC value (µl/ml)				
E. coli	25	0	50	12.5	6.25
B. subtilis	25	0	50	3.125	3.125
S. aureus	100	0	50	6.25	6.25
S. pyogenes	50	0	50	3.125	6.25

Table 2. The chemical composition of mint essential oil analyzed by GC-MS

Mint essential oil				
No.	Compounds	RI	Rlit	%
1	Limonene	1027	1027	2.1
2	Eucalyptol	1033	1034	5.9
3	Menthone	1150	1151	11.2
4	Menthol	1173	1173	68.9
5	α Terpineol	1189	1189	0.3
6	Isomenthol	1448	1449	0.45
7	Germacrene D	1482	1483	1.5
8	Germacrene D-4-ol	1570	1572	0.9
	Total (%)			91.25

Table 3. The chemical composition of myrrh essential oil analyzed by GCMS

Myrrh essential oil				
No.	Compounds	RI	Rlit	%
1	Monogene	1019	1020	2.9
2	Menthofuran	1162	1163	3.5
3	β elemene	1380	1381	2.5
4	Caryophyllene	1423	1423	18.3
5	Curzerene	1478	1478	45.9
6	Cadinene	1500	1502	3.1
7	Germacrene B	1550	1551	3.2
8	Furanoeudesma 1,3-diene	1585	1587	20.1
	Total (%)			99.5

The tested essential oils were observed effective in inhibiting the growth of tested pathogenic bacteria however there was a variation in the level of pathogen susceptibility to the oils. The findings from this study highlight the significant antibacterial potential of mint and myrrh essential oils against all four tested bacteria. The efficacy of these oils, as demonstrated by their low minimum inhibitory concentrations (MIC) and substantial zones of inhibition, aligns with recent literature emphasizing the growing importance of plant-based antimicrobials in combating bacterial infections, especially antibiotic-resistant strains. Hydro distilled essential oil of *Mentha* species were reported earlier for their antibacterial activity against *S. aureus*, *B. subtilis*, *S. pyogenes, E. coli, P. aeruginosa,* and *A. baumannii*[8,10]. The antibacterial activity of hydro distilled myrrh essential oil has been reported previously[11,17]. Overall the MIC values of the tested essential oil ranges between 3.125- 100 µl/ml. The MIC value of 0.25% to ≥2% v/v was observed for the oil thyme and oregon[15]. Arugula oil has been reported to possess antibacterial activity with the MIC value range 50-72 µg/mL[18]. A study recorded the MIC value of essential oil of *M. arvensis* as 25.0, 12.5 and 12.5 l/ml for the *B. subtilis, S. aureus* and *E. coli,* respectively[10]. Myrrh essential oils showed MIC values ranged between 1.8 and 17.2 mg/ml against gram positive and negative bacteria[19,20,21].

Thyme and cinnamon oils demonstrated moderate antibacterial effects, suggesting that while they possess antimicrobial properties, their efficacy may depend on specific bacterial strains and oil concentrations. Arugula oil's weak antibacterial activity indicates a limited role in therapeutic applications but warrants further investigation for synergistic effects with other oils[21]. The chemical composition of these essential oils likely plays a crucial role in their antimicrobial activities. Mint essential oil's high menthol content corresponds with previous studies, which have identified menthol as a key compound with potent antimicrobial and anti-inflammatory properties[22,23]. Similarly, the dominance of curzerene in myrrh oil corroborates findings from recent research that links this compound with strong antibacterial and anti-inflammatory effects[13,17]. Both oils have been shown to outperform traditional antibiotics like ciprofloxacin in some cases, which is significant in the context of rising antibiotic resistance.

The variation in the antibacterial efficacy of essential oils can be attributed to differences in their chemical compositions. Compounds such as terpenes, monoterpene hydrocarbons, and oxygenated monoterpenes present in essential oils have been well-documented for their microbial toxicity [20,24]. Although the precise mechanism of action remains unclear, a key property of essential oils is their hydrophobic nature, which allows them to interact with and disrupt bacterial cell membranes. Mint oil's high menthol content (68.9%) likely contributes to its strong antibacterial action, as menthol disrupts bacterial cell membranes, leading to cell lysis[8]. This disruption leads to the leakage of vital cellular components, including ions and molecules, which compromises essential processes like ion transport and respiration, ultimately causing cell death[12]. Similarly, curzerene in myrrh oil may inhibit bacterial growth by interfering with cellular processes and metabolic pathways. The ability of mint and myrrh oils to outperform ciprofloxacin against certain pathogens (*B. subtilis, S. aureus, and S. pyogenes*) underscores their potential to fill gaps where conventional antibiotics fail, particularly against resistant strains. These findings suggest that essential oils could be beneficial in reducing bacterial contamination when incorporated into food products or when combined with antibiotics to enhance their efficacy through synergistic effects[18,19]. This dual approach could help in addressing food safety concerns and combating the growing issue of antibiotic resistance.

Conclusions

The potent antibacterial activities of mint and myrrh essential oils, as revealed in this study, support their potential use as alternative treatments for bacterial infections, particularly in the context of rising resistance to conventional antibiotics. Further research should focus on evaluating the efficacy of mint and myrrh oils in vivo, assessing their potential as therapeutic agents for treating bacterial infections in humans. Detailed investigations are required to elucidate the exact mechanisms of action of the bioactive compounds, such as menthol and curzerene, against bacterial cells. Exploring the synergistic effects of mint and myrrh oils with conventional antibiotics or other natural compounds could enhance their antibacterial potency and broaden their spectrum of activity. Beyond healthcare, the antibacterial properties of these oils hold promise for applications in food preservation, agriculture, and cosmetics, warranting further exploration in these domains.

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Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could influence the work reported in this paper.

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