https://doi.org/10.31925/farmacia.2025.2.13 **ORIGINAL ARTICLE**

NEW PERSPECTIVES IN THE ANTIMICROBIAL POTENTIAL OF THYME AND OREGANO ESSENTIAL OILS

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Abstract

The present study investigates the antimicrobial activity of oregano (*Origanum vulgare* L.) and thyme (*Thymus vulgaris* L.) essential oils against seven clinically relevant microbial strains, including Gram-positive, Gram-negative bacteria, and *Candida albicans*. Using gas chromatography, the chemical composition of both oils was identified, with carvacrol being the major compound in oregano oil (81.20%) and thymol dominating thyme oil (41.84%). Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) were determined. Oregano oil demonstrated stronger antimicrobial efficacy, particularly against Gram-negative strains such as *E. coli* and *P. aeruginosa*, while thyme oil showed higher potency against Gram-positive *Bacillus spizizenii*. The results confirm the potential use of these essential oils as natural antimicrobial agents, either alone or in combination with conventional antibiotics, contributing to combatting antimicrobial resistance and offering possible applications in medical and food preservation fields.

Rezumat

Acest studiu a investigat activitatea antimicrobiană a uleiurilor volatile de oregano (*Origanum vulgare* L.) și cimbru (*Thymus vulgaris* L.) asupra unui număr de șapte tulpini microbiene relevante din punct de vedere clinic, incluzând bacterii Gram-pozitive, Gram-negative și *Candida albicans***.** Compoziția chimică a celor două uleiuri a fost determinată prin gaz-cromatografie, evidențiindu-se carvacrolul ca principal compus în uleiul de oregano (81,20%) și timolul în uleiul de cimbru (41,84%). Au fost stabilite concentrațiile minime inhibitorii (MIC) și concentrațiile minime bactericide (MBC). Uleiul de oregano a prezentat o eficiență antimicrobiană superioară, în special împotriva bacteriilor Gram-negative precum *E. coli* și *P. aeruginosa*, în timp ce uleiul de cimbru s-a dovedit mai eficient împotriva bacteriei Gram-pozitive *Bacillus spizizenii*. Rezultatele confirmă potențialul acestor uleiuri esențiale ca agenți antimicrobieni naturali, fie individual, fie în combinație cu antibiotice convenționale, contribuind la combaterea rezistenței antimicrobiene și deschizând perspective pentru aplicații în domeniul medical și al conservării alimentelor.

Keywords: antimicrobial; oregano; thyme; essential oils

Introduction

Essential oils (EOs) are aromatic extracts obtained from various plant parts. Their composition is complex and varies depending on the plant species from which they are extracted, with over 200 chemical compounds found in some. In recent years, essential oils have become an important resource in fields such as perfumery, cosmetics, pharmacy, and traditional medicine due to their antibacterial, antiviral, fungicidal and antioxidant capabilities [1, 2].

A relationship between the strength of the antimicrobial properties and the constituent components has been established as follows: phenols > aldehydes > ketones > alcohols > esters > hydrocarbons [3]. However, the use of only the main components of an essential oil has been shown to have a lower antimicrobial effect than the use of the whole oil, suggesting important synergistic effects between all components that can influence multiple biochemical processes in micro-organisms [4]. Antimicrobial resistance has been recognised by the WHO as one of the ten leading health problems at the global level, highlighting the need for alternative antimicrobial strategies [5]. A key property of essential oils and their components is their hydrophobic nature, enabling them to integrate with the lipids in bacterial and mitochondrial cell membranes. This process disrupts the cell structures, increasing membrane permeability [6].

Compounds such as thymol, carvacrol and eugenol have demonstrated fungicidal activity and EOs rich in these components have been shown to exhibit the highest inhibitory activity against various pathogens [7, 8]. Thymol and carvacrol are monoterpenes present in many EOs, including those from oregano (*Origanum vulgare* L.) (OEO) and thyme (*Thymus vulgaris* L.) (TEO) [9].

Karaday M *et al*., presented a review of the antimicrobial activity of carvacrol against *Bacillus cereus*. According to their findings, carvacrol causes loss of the ion gradient in the target cell by depleting the intracellular ATP pool, altering the membrane potential, and increasing the permeability of the cytoplasmic membrane to protons and potassium ions [10]. These critical changes ultimately lead to the death of the target cell [11]. Results from another study showed that OEO has antimicrobial effects on *A. baumannii*-MDR by destabilising and disrupting the cell membrane [12]. In addition to antimicrobial effects, oregano oil and carvacrol have been shown to inhibit certain genes, particularly virulence genes in enterohemorrhagic *E. coli* [10, 13]. Carvacrol can interfere with the activity of cell wall enzymes, thereby inhibiting bacterial growth by damaging the bacterial cell wall. The possible mechanism of action of carvacrol against *Streptococcus pyogenes* according to Wijesundara NM *et al*., (2021) is through the induction of morphological changes, cytoplasmic leakage and, therefore, cell damage [14]. Carvacrol extracted from *T. zygis* L. has been found to be effective against several bacterial strains, including *S. aureus*, *E. coli* and *Pseudomonas aeruginosa* [15, 16].
Thymol is a structural isomer of carvacrol and is one of the main constituents of thyme oil. It has been studied the most and has been shown to exert a range of therapeutic properties, including antimicrobial, anti-tumour, antifungal, anti-parasitic, antioxidant and anti-inflammatory. P-cymene is another compound present in TEO in relatively high concentrations with antiviral, antioxidant and antitumor properties, especially when conjugated with ruthenium [16-20]. γ-Terpinene is another predominant constituent in TEO, which belongs to the monoterpene group and has been shown to inhibit the growth of helminth and protozoan infections, but only as a component in Australian tea tree oil [21]. (E)-β-Caryophellene is also a constituent of TEO with anti-inflammatory and pro-apoptotic properties in tumour cells [22]. Similar to thymol and carvacrol, (E)-β-Caryophyllene also exhibits antibacterial properties [23]. Thymol has demonstrated effectiveness against both gram-positive and gram-negative bacteria. Thoshar *et al*. evaluated various phenol-rich essential oils, including thyme oil, for their minimal inhibitory concentration (MIC) and found that a concentration of 2 µL/mL was effective against *E. coli*. In contrast, higher concentrations were necessary to inhibit the growth of *E. faecalis* and *S. aureus* [24]. Thyme oil, high in thymol, was also tested for its antibacterial effects against methicillin-resistant *S. aureus* (MRSA) strains. Kryvtsova *et al*. studied Thyme oil from *T. vulgaris* for antimicrobial action against MRSA strains isolated from patients with periodontitis and pharyngitis; results showed that thyme oil concentrations of 0.01% and 0.05% inhibited bacterial growth by 53% and 76%, respectively. In this study, phenolic monoterpenes like thymol were the primary components of the volatile oil [25]. Additionally, Tohidpour *et al*. found thymol extracted from *T. vulgaris* to be more effective than *Eucalyptus globulus* essential oil in limiting MRSA growth [26].
The present article aims to compare the antimicrobial properties of oregano and thyme essential oils by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against significant clinical pathogens. This study provides a comparative evaluation of the antimicrobial activity of oregano and thyme essential oils, assessing both minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) to distinguish between bacteriostatic and bactericidal effects. In contrast to previous research, we integrate statistical validation to provide a more precise and reliable assessment of their potential applications.

Materials and Methods

The thyme and oregano essential oils used in this study ware purchased from the commercial market and is 100% pure with certified organic ingredients. For the purposes of their chemical analysis, an apparatus was used, a gas chromatographic system, consisting of a 7890A gas chromatograph, a flame ionization detector and a mass spectral detector 5975C (Agilent Technologies); column Stabilwax (Restek) with parameters: length 30m, diameter 0.25 mm and film coating thickness 0.25 µm at the following temperature program: initial temperature 65°C, increased to 170°C at a rate of 1.5°C/min; analysis duration 70 min; injector and detector temperatures 250°C, FID temperature: 250°C; carrier gas hydrogen with a flow rate of 0.8 mL/min; carrier gas helium with a flow rate of 0.8 mL/min; scanning range of the mass spectral detector m/z = 40 - 450; injected sample volume 1.0 µl in 100:1 flow split mode. Compounds were identified by comparing retention times and relative Kovats indices (RI) with those of standard substances and mass spectral data from the NIST'08 (National Institute of Standards and Technology, USA) and Adams Libraries.
The antimicrobial properties of OEO and TEO were examined against seven microbial strains - *Esherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, *Streptococcus pyogenes* ATCC 12384, *Bacillus spizizenii* ATCC 6633 and *Candida albicans* ATCC 10231. For the purposes of the study, 1% DMSO solutions of the essential oils were prepared in concentrations ranging from 0.15% (v/v) - 5% (v/v). The antimicrobial activity test was conducted in Brain Heart Infusion (BHI) broth and on Blood agar (HiMedia), provided by

Ridacom, Bulgaria. As a negative control for all samples, a 1% DMSO solution was used.

Minimum inhibitory concentration (MIC) determination

We used the two-fold serial dilution method to determine the MICs. For all microbial strains, we used a row of six tubes with 1 ml of BHI broth, in which we successively diluted each of the EOs to concentrations of 0.15% (v/v) to 5% (v/v). Then, in each tube, we placed 0.1 mL of a 24-hour standardized (0.5 MF) microbial suspension of the respective test strain. All samples were prepared in triplicate and at the same time we placed both positive controls (1 mL of BHI broth with 0.1 mL of suspension of the respective microbe) and negative controls (1 mL of BHI broth with 1 mL of the respective EO, and after mixing, 1 mL of suspension was removed from the test tube). All samples were incubated aerobically: bacterial strains were incubated for 24 hours at 37°C, while the *C. albicans* strain was incubated for 48 hours at 35°C. The MICs were determined as the highest dilutions at which microbial growth was completely inhibited, indicated by the absence of turbidity in the suspension.

Minimum bactericidal concentration (MBC) determination

In our study, determined the minimum bactericidal concentrations (MBCs) by aliquoting the microbial suspension from all MIC test tubes and plating it on blood agar. All samples were incubated aerobically: bacterial strains were incubated for 24 hours at 37°C, while the *C. albicans* strain was incubated for 48 hours at 35°C. The MBCs were defined as the highest dilutions at which microbial growth was inhibited by 99.9%.

Statistical Analysis

The data were expressed as means ± standard deviation. The mean separations were analysed using Student's t-test and one-way analysis of variance (One-Way ANOVA) using Tukey's test; Differences were considered statistically significant at $P < 0.05$.

Results and Discussion

In our study, 22 compounds were identified in OEO and 21 compounds in TEO. The main components in OEO, present in the highest concentrations, include carvacrol (81.20%), p-cymene (3.69%), ɣ-terpinene (3.52%). In TEO, the primary components are thymol (41.84%), p-cymene (19.85%), ɣ-terpinene (12.64%), β-Caryophyllene (2.88%) and Carvacrol (2.14%). From the perspective of the antimicrobial activity examined, the relative percentage content of these main components is of critical importance. The chemical compositions of oregano and thyme essential oils are shown in Table I.

Table I

Chemical compositions of oregano and thyme EOs

No. Compound	Retention time (min)	% of total ion current	Retention time (min)	% of total ion current
	Thyme EO		Oregano EO	
α-Thujene	9.11	1.38	9.05	0.12
α-Pinene	9.32	1.32	9.27	0.43
Camphene	9.84	1.19	9.79	0.16
β-Pinene	10.75	0.76	10.71	0.08
β-Myrcene	11.23	0.88	11.15	0.64
α- Phellandrene	-	-	11.16	0.11
α-Terpinene	12.06	1.18	12.02	0.90
p-Cymene	12.31	19.85	12.28	3.69
Limonene	12.47	0.80	12.40	0.13
β - Phellandrene	-	-	12.45	0.21
ɣ-Terpinene	13.41	12.64	13.37	3.52
Sabinene hydrate	13.79	0.57	13.73	0.16
β-Linalool	14.74	2.03	14.73	3.76
Camphor	16.12	1.17	-	-
Terpinolen	-	-	14.25	0.10
Borneol	16.90	1.81	16.87	0.71
α-Terpineol	-	-	17.60	0.24
Terpinen-4-ol	17.16	0.84	-	-
Thymol methyl ether	18.58	0.31	-	-
Carvacrol, methyl ether	18.84	1.39	-	-
Thymol	20.37	41.84	20.36	1.19
Carvacrol	20.75	2.14	20.75	81.20
β-Caryophyllene	23.77	2.88	23.70	0.97
Aromadendrene	-	-	24.18	0.15
β-Bisabolene	-	-	25.93	1.24
δ-Cadinene	26.19	0.64	-	-
Caryophyllene oxide	27.73	0.58	27.66	0.17

We investigated the antimicrobial activities of EO and TEO by applying various concentrations (0.15% (v/v) to 5% (v/v)) in Brain Heart Infusion (BHI) broth against seven microbial strains relevant to clinical practice in human medicine.

Using the serial dilution method to determine MICs, we found that OEO and TEO exhibited antimicrobial activity against all tested strains - Gram-negative (*E. coli* ATCC 25922, *K. pneumoniae* ATCC 13883 and *P. aeruginosa* ATCC 27853), Gram-positive (*S. aureus* ATCC 29213, *Str. pyogenes* ATCC 12384 and *B. spizizenii* ATCC 6633) and fungi *C. albicans* ATCC 10231. The results are presented in Table II.

Table II

Antibacterial activity of oregano essential oil (OEO) and тhyme essential oil (TEO) against microbial ATCC strains by MICs and MBCs determination

	OEO		**TEO**		**DMSO 1%**
Microbial strains	**MICs (v/v)**	**MBCs (v/v)**	**MICs (v/v)**	**MBCs (v/v)**	
***E. coli* ATCC 25922**	0.31 **SD ± 0.004**	0.31 **SD ± 0.0006**	1.25 **SD ± 0.001**	1.25 **SD ± 0.001**	**Negative control**
***K. pneumoniae* ATCC 13883**	0.62 **SD ± 0.002**	0.62 **SD ± 0.001**	0.62 **SD ± 0.001**	0.62 **SD ± 0.0006**	
***P. aeruginosa* ATCC 27853**	1.25 **SD ± 0.015**	1.25 **SD ± 0.023**	2.5 **SD ± 0.1**	5 **SD ± 0.15**	
***S. aureus* ATCC 29213**	0.62 **SD ± 0.003**	> 5 **SD ± 0.47**	0.62 **SD ± 0.002**	> 5 **SD ± 0.61**	
***Str. pyogenes* ATCC 12384**	0.62 **SD ± 0.002**	> 5 **SD ± 0.1**	1.25 **SD ± 0.0006**	5 **SD ± 0.15**	
***B. spizizenii* ATCC 6633**	0.62 **SD ± 0.001**	0.62 **SD ± 0.003**	0.31 **SD ± 0.001**	0.31 **SD ± 0.004**	
***C. albicans* ATCC 10231**	0.62 **SD ± 0.001**	> 5 **SD ± 0.44**	1.25 **SD ± 0.001**	> 5 **SD ± 0.4**	

For the antimicrobial effects of OEO against the majority of the tested microbial strains (5/7), we found a MIC of 0.62% (v/v) against *K. pneumoniae*, *S. aureus*, *Streptococcus pyogenes*, *B. spizizenii* and *C. albicans.* For *E. coli* the OEO demonstrated MIC at a lower concentration - 0.31% (v/v), and higher against *P. aeruginosa* - 1.25% (v/v).

TEO also exhibited antimicrobial activity against all tested microbes, though for nearly half of them (4/7), MIC values were higher compared to OEO. Against *B. spizizenii*, however, the antimicrobial activity of TEO was higher than that of OEO - MIC = 0.31% (v/v). The MICs for TEO were determined as 0.62% (v/v) for *K. pneumoniae* ATCC 13883 and *S. aureus* ATCC 29213. For *E. coli* ATCC 25922, *Str. pyogenes* ATCC 12384 and C. *albicans* ATCC 10231 the MICs values were 1.25% (v/v), and for *P. aeruginosa* ATCC 27853 were the highest - MIC = 2.5% (v/v).

Following the MIC results, blood agar cultures were prepared from all test samples to determine the MBCs (Table II). The MBCs values for EEO against *E. coli*, *K. pneumoniae*, *P. aeruginosa* and *B. spizizenii* were identical to the MICs already established for the respective microorganism. For *E. coli* we determined MBC = 0.31% (v/v), *K. pneumoniae* and *B. spizizenii* – MBC = 0.62% (v/v) and against *P. aeruginosa* – MBC = 1.25% (v/v).

For the remaining test strains – *S. aureus*, *S. pyogenes* and *C. albicans* – no MBC/MFC values for OEO were detected. It is likely that the concentrations of EOs that completely inhibited the growth of these three strains were higher than those tested by us, *i.e.* > 5% (v/v). This suggests that, in relation to these microbes, OEO primarily exhibits a bacteriostatic effect rather than a pronounced bactericidal effect.

For TEO, the lowest MBCs were observed for *B. spizizenii*, *K. pneumoniae* and *E. coli* – 0.31% (v/v), 0.62% (v/v), and 1.25% (v/v), respectively. The MBCs of TEO against *P. aeruginosa* and *S. pyogenes* were 5% (v/v), while for *S. aureus* and *C. albicans*, no MBC/MFC values could be determined.

The statistical analysis of the antibacterial activity of the two essential oils was conducted by evaluating their minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) against the specified pathogens. The significance of differences in their antimicrobial potential was assessed using appropriate statistical methods. The results of the analysis are presented in Table III.

The statistical comparison of MIC and MBC values revealed significant differences in the efficacy of the two essential oils against certain pathogens, as indicated by the p-values.

Table III

Statistical data of MIC and MBC of oregano and thyme EOs

Microbial strains	**OEO MICs (v/v)**	**TEO MICs (v/v)**	**p-value**	**OEO MBCs (v/v)**	**TEOMBCs (v/v)**	**p-value**
***E. coli* ATCC 25922**	0.31 **SD ± 0.004**	1.25 **SD ± 0.001**	**P < 0.05**	0.31 **SD ± 0.0006**	1.25 **SD ± 0.001**	**P < 0.05**

Microbial strains	OEO MICs (v/v)	TEO MICs (v/v)	p-value	OEO MBCs (v/v)	TEOMBCs (v/v)	p-value
***K. pneumoniae* ATCC 13883**	0.62 **SD ± 0.002**	0.62 **SD ± 0.001**	**P = 1**	0.62 **SD ± 0.001**	0.62 **SD ± 0.0006**	**P = 1**
***P. aeruginosa* ATCC 27853**	1.25 **SD ± 0.015**	2.5 **SD ± 0.1**	**P < 0.05**	1.25 **SD ± 0.023**	5 **SD ± 0.15**	**P < 0.05**
***S. aureus* ATCC 29213**	0.62 **SD ± 0.003**	0.62 **SD ± 0.002**	**P = 1**	> 5 **SD ± 0.47**	>5 **SD ± 0.61**	**P = 1**
***Str. pyogenes* ATCC 12384**	0.62 **SD ± 0.002**	1.25 **SD ± 0.0006**	**P < 0.05**	> 5 **SD ± 0.1**	5 **SD ± 0.15**	**P = 1**
***B. spizizenii* ATCC 6633**	0.62 **SD ± 0.001**	0.31 **SD ± 0.001**	**P < 0.05**	0.62 **SD ± 0.003**	0.31 **SD ± 0.004**	**P < 0.05**
***C. albicans* ATCC 10231**	0.62 **SD ± 0.001**	1.25 **SD ± 0.001**	**P < 0.05**	> 5 **SD ± 0.44**	>5 **SD ± 0.4**	**P = 1**

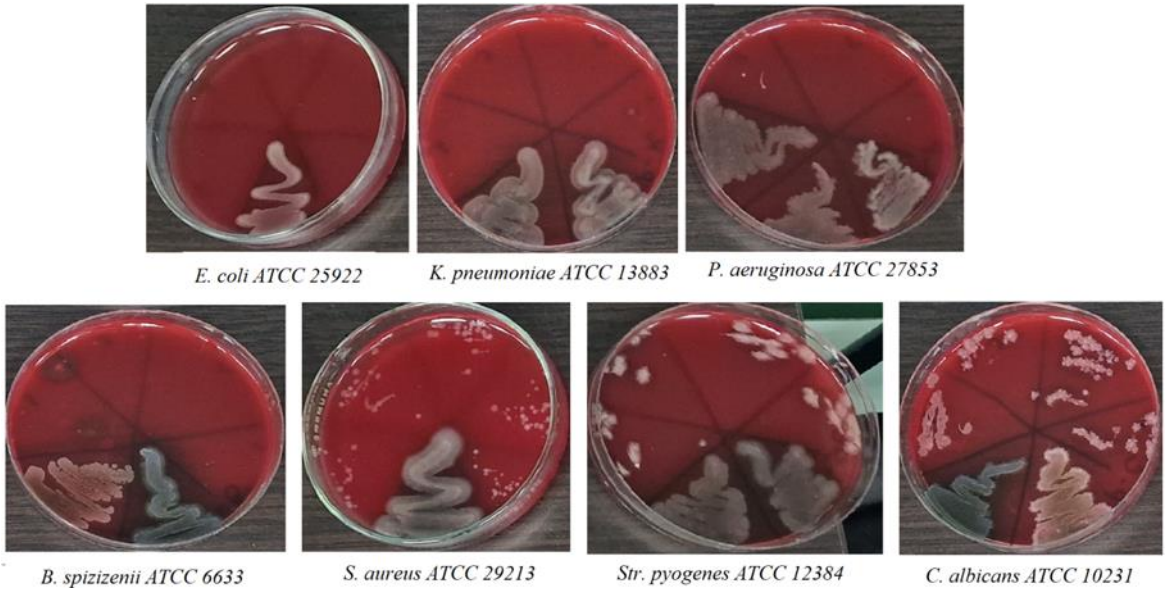

Figure 1.
Determination of MBCs/MFC (concentration range 0.15 - 5% (v/v)) values for Oregano essential oil against ATCC strains

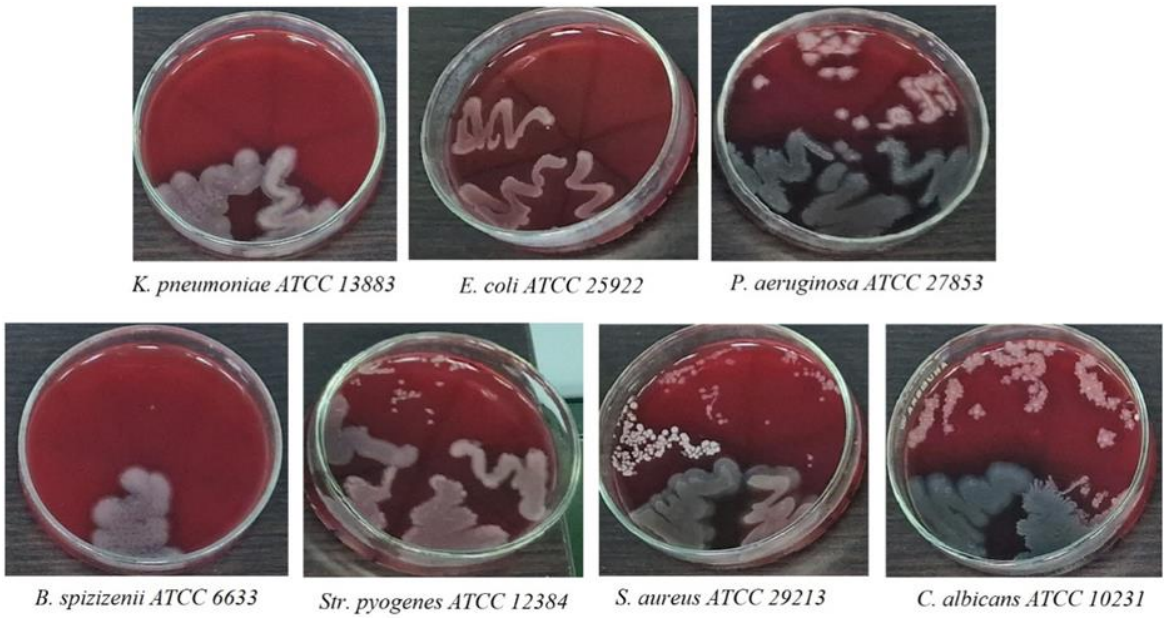

Figure 2.
Determination of MBCs/MFC (concentration range 0.15 - 5% (v/v)) values for Thyme essential oil against ATCC strains

Gram-negative bacteria

Escherichia coli ATCC 25922 was significantly more susceptible to OEO (MIC = 0.31% v/v) compared to THEO (MIC = 1.25% v/v, $p < 0.05$). The same trend was observed for the MBC values, suggesting that OEO exhibits stronger bactericidal activity against *E. coli*. *Klebsiella pneumoniae* ATCC 13883 showed equal susceptibility to both essential oils (MIC and MBC = 0.62% v/v, $p = 1$), indicating comparable antimicrobial efficacy. *Pseudomonas aeruginosa* ATCC 27853 was more resistant to THEO, as evidenced by significantly higher MIC (2.5% v/v) and MBC (5% v/v) values compared to OEO (MIC and MBC = 1.25% v/v, $p < 0.05$). This suggests that OEO is more effective against *P. aeruginosa*.

Gram-positive bacteria

Staphylococcus aureus ATCC 29213 exhibited equal MIC values for both oils (0.62% v/v, $p = 1$), but the MBC values exceeded 5% v/v for both oils, indicating only bacteriostatic effects at the tested concentrations. *Streptococcus pyogenes* ATCC 12384 was more susceptible to OEO (MIC = 0.62% v/v) than to THEO (MIC = 1.25% v/v, $p < 0.05$). However, both oils exhibited MBC values >5% v/v ($p = 1$), indicating limited bactericidal activity. *Bacillus spizizenii* ATCC 6633 showed significantly higher susceptibility to THEO (MIC = 0.31% v/v) compared to OEO (MIC = 0.62% v/v, $p < 0.05$). The same trend was observed for MBC values, suggesting that THEO has stronger antimicrobial activity against *B. spizizenii*.

Fungal strain

Candida albicans ATCC 10231 exhibited significantly lower MIC values for OEO (0.62% v/v) compared to THEO (1.25% v/v, $p < 0.05$), suggesting that OEO has a stronger inhibitory effect. However, both oils showed MBC values exceeding 5% v/v ($p = 1$), indicating a fungistatic rather than fungicidal effect at the tested concentrations.

Essential oils have been used since ancient times in aromatherapy and in the treatment and prevention of various diseases, often empirically, without knowledge of their precise targets in microorganisms. One of their most pronounced effects is their antimicrobial activity, particularly in essential oils from the *Lamiaceae* family. Oregano and thyme essential oils are among the most frequently studied due to their well-documented antimicrobial properties, largely attributed to their two main components – carvacrol and thymol. Carvacrol has demonstrated significant antibacterial activity, primarily through permeabilisation and depolarisation of the cytoplasmic membrane [27, 28]. Additionally, it inhibits microbial toxin production and biofilm formation, reduces fimbriae production and motility of uropathogenic *E. coli*, and exhibits anti-inflammatory effects [29, 30]. Essential oils with higher concentrations of carvacrol and thymol have been shown to exert stronger antimicrobial effects [31]. Thyme and oregano essential oils have been tested against *E. coli* O157:H7 isolated from bovine faeces [32]. Thyme essential oil has also demonstrated activity against both antifungal-sensitive and antifungal-resistant yeast strains [33]. The antimicrobial properties of carvacrol and thymol are closely related to their molecular structures. The only structural difference between them is the position of the hydroxyl group, which significantly influences their mechanism of action. This, along with variations in terpene concentrations in different essential oils, likely explains differences in their antimicrobial activity [34]. Due to this structural difference, carvacrol is more effective at inhibiting biofilm formation in *Pseudomonas* spp. [35]. Additionally, both carvacrol and thymol disrupt bacterial cell membranes, increase permeability and reduce cytoplasmic pH in *Pseudomonas aeruginosa* and *Staphylococcus aureus* [36]. Our results clearly demonstrate high activity against Gram-positive and Gram-negative bacteria. Gram-negative bacteria are generally more resistant due to their complex outer membrane, which can hinder the action of natural compounds. However, in our study, *E. coli* showed significant susceptibility to OEO. This effect is likely due to the ability of carvacrol and thymol to degrade the outer membrane of Gram-negative bacteria, leading to lipopolysaccharide release and increased permeability for ATP loss. Regarding Gram-positive *S. aureus*, OEO induces phosphate ion efflux, contributing to its antibacterial activity [36]. The antimicrobial effects of thyme essential oil have also been extensively documented, with reports indicating inhibition of various Gram-positive and Gram-negative bacteria [37, 38, 39]. The fungicidal effect we observed is also significant, especially in the two forms for internal use of the two oils (oregano and thyme). The reported MIC and MBC values of 1 µg/mL were superior to those found by Karpiński *et al*. [40]. The concentration ranges of essential oils used in our study were determined based on our previous findings [41, 42] as well as other published studies [43, 44]. According to the data of Sateriale *et al*., and in other studies [45] results were similar across the bacterial strains presented in our study [46]. According to data of other authors MICs of WEEE against *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *Str. pyogenes* and *C. albicans* may also demonstrate lower values in the range 0.03 - 0.16% (v/v) [43, 47, 48]. The MIC of OEO against *B. spizizenii* was consistent with previous findings [44]. Alkhafaji and Jayashankar [49] reported that oregano essential oil exhibits antimicrobial activity against a wide range of pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Enterococcus faecalis*, *Acinetobacter baumannii*, *Neisseria gonorrhoeae*, *Staphylococcus aureus* and *Staphylococcus epidermidis*. This suggests its potential for medical applications, particularly as an alternative or adjunct antimicrobial agent [50].

According to a review by Soleimani *et al*., MIC values of essential oils from *T. vulgaris* and *T. mastichina* vary widely, typically ranging between 0.02% (v/v) and 2.4% (v/v) against the microbial strains investigated in this study [51]. However, for *S. aureus* ATCC 29213 [44,52], *C. albicans* ATCC 10231 [52], and *S. pyogenes* ATCC 12384 [53], MBC/MFC values have been reported in a broad range between 0.246 and 250 mg/mL. Maggini *et al*. [54] suggested that oregano essential oil (OEO), particularly those rich in carvacrol and thymol and containing significant amounts of monoterpene hydrocarbons, could serve as a promising standardized herbal antimicrobial product. In a study by Dolores Ibáñez *et al*. [55], OEO and its primary compound, carvacrol, effectively inhibited bacterial growth at minimal doses (1 μL). Carvacrol alone demonstrated antimicrobial effects against *S. aureus, P. aeruginosa*, coagulase-negative *Staphylococcus spp*., *Salmonella spp.*, *Enterococcus spp.*, *Shigella spp*. and *E. coli*. Furthermore, both carvacrol and thymol exhibited activity against *Salmonella enterica* and *S. aureus* [56].

Thyme and oregano essential oils (TEO and OEO) are proven antimicrobial agents with effectiveness against a range of pathogens, including antibiotic-resistant bacteria, and exhibit inhibitory properties against bacterial biofilms [57]. Conducted disk diffusion tests confirm their strong antibacterial activity, particularly when combined with antibiotics such as tetracycline, highlighting their potential clinical application [58]. Additionally, cytotoxicity studies indicate a dose-dependent effect on human cells, emphasizing the need for further safety assessments before clinical use [58]. Moreover, their broad spectrum of biological properties, including antioxidant, anti-inflammatory and antitumor effects, supports their potential for broader applications in medicine and pharmaceuticals [59].

The statistical analysis of MIC and MBC values for OEO and TEO revealed significant differences in antimicrobial efficacy, particularly against Gram-negative bacteria such as *E. coli* and *P. aeruginosa*. This underscores the importance of considering the specific chemical composition of essential oils when evaluating their potential antimicrobial applications. Our findings highlight that oregano oil is more effective against Gram-negative bacteria, while thyme oil shows greater potency against specific Gram-positive strains, emphasizing the role of chemical composition in antimicrobial specificity. This study advances previous research by providing a direct MIC *vs*. MBC comparison and statistical validation, strengthening its relevance for future clinical applications [60].

Given the increasing challenge of antibiotic resistance, the strong antimicrobial activity of OEO and TEO suggests their potential use as natural alternatives or adjuncts to conventional antibiotics [61]. Unlike broad-spectrum antibiotics, which can disrupt the natural microbiota and contribute to resistance, these essential oils offer a more targeted antimicrobial approach that may help preserve microbial balance while effectively combating bacterial infections.

Conclusions

In conclusion, both OEO and TEO demonstrated significant antimicrobial activity, with notable differences in efficacy against different microbial strains. These oils show promise for medical applications, particularly in combating antibiotic-resistant pathogens and in the development of natural preservatives for food safety. Given the growing concerns over antimicrobial resistance (AMR), OEO and TEO present a valuable natural alternative or complement to conventional antibiotics. To enhance their practical application, future studies should focus on optimising their formulations, determining safe dosage levels for clinical use, and evaluating their effectiveness in *in vivo* models. These findings highlight the potential of OEO and TEO as natural antimicrobial agents, suggesting their promising use in medical applications, pharmaceutical formulations, and food preservation strategies. Additionally, investigating their potential synergistic effects with existing antibiotics and assessing their role in biofilm inhibition could further validate their therapeutic potential.

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Conflict of interest

The authors declare no conflict of interest.

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