İSTANBUL UNIVERSITY PRESS

Turkish Journal of Bioscience and Collections,
TJBC 2025, 9 (1): 7–18

https://doi.org/10.26650/tjbc.1624479

Submitted 21.01.2025
Revision Requested 10.02.2025
Last Revision Received 02.03.2025
Accepted 03.03.2025
Published Online 12.03.2025

Turkish Journal of Bioscience and Collections

Research Article

Open Access

Antimicrobial Activity of Essential Oils for Potential Use as Wound Dressing Material Additives

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Abstract

Objective: The use of natural products in wound care has a long history, with herbal extracts and aromatic oils offering therapeutic benefits such as antimicrobial and antioxidant properties. This study investigated the antimicrobial efficacy of essential oils (tea tree, thyme, and cinnamon) incorporated into non-woven, hypoallergenic, and sterile wound dressings.

Materials and Methods: The antimicrobial activity of tea tree, thyme, and cinnamon oils was evaluated using minimum inhibitory concentration (MIC) and minimum bactericidal/fungicidal concentration (MBC/MFC) tests. After application of the MBC doses determined in our study to the wound dressing and gauze, the samples were evaluated using a stepwise qualitative to quantitative experimental design to assess the static and cidal effects.

Results: Tea tree oil showed the highest MIC/MBC values (1-3 mg/mL), while thyme and cinnamon oils showed efficacy at lower concentrations (0.25 mg/mL) against *Staphylococcus aureus* and *Escherichia coli*. In addition, the essential oils showed significant antifungal activity against *Candida albicans* at lower concentrations than bacteria. The impregnated dressings exhibited strong antimicrobial properties with a 99.99% (4 log) reduction in microbial growth, confirming the potential of essential oils as viable, biocompatible alternatives to traditional chemical agents in wound healing applications.

Conclusion: These findings highlight the benefits of natural essential oils in improving wound care while minimising the risk of toxicity and resistance associated with chemical treatments. In conclusion, based on the results of our study, wound dressings with natural essential oils used to reduce the risk of infection and indirectly promote wound healing can reduce healing time and improve patient quality of life in a cost-effective care setting.

Keywords Aromatic oils · Bioactive compounds · Infection control · Natural antimicrobial agents · Phytochemicals

Citation: Karakuş, B. & Şanlı, N. Ö. (2025). Antimicrobial activity of essential oils for potential use as wound dressing material additives. *Turkish Journal of Bioscience and Collections*, *9*(1), 7-18. https://doi.org/10.26650/tjbc.1624479

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Introduction

Essential oils are volatile and aromatic compounds derived from the secondary metabolism of plants. They are produced in glandular trichomes located in various leaves and stems as a result of the plant's interaction with its natural environment (Hili, 1997). The application of these oils as antimicrobial agents has been advocated for many years. Notably, there is a growing trend towards traditional therapeutic methods to obtain alternative and more effective products, driven by the increasing reliance on synthetic and/or semi-synthetic antimicrobial agents, which has contributed to the emergence of antimicrobial resistance (Goel *et al*., 2016).

It is well-established that microorganisms within the skin flora, which facilitate interaction with the external environment, can become opportunistic pathogens due to various factors, including an increase in their population, changes in their location, or alterations in the conditions to which they are exposed (Çitil *et al*., 2015). When the integrity of the skin is compromised, wounds create an entry point for infections. This is particularly concerning for individuals with weakened immune systems or impaired blood circulation, such as cancer patients or those with diabetes, as wound healing can be significantly prolonged in these populations. Rapid healing of wounds and restoration of skin integrity are essential. It is well-documented that mechanical barriers, such as wound dressings and gauze, are insufficient on their own, as they cannot effectively prevent the passage of bacteria (Türsen, 2013). The objective is to isolate chronic wound infections, which can develop into skin lesions or ulcers, from the external environment using barriers such as alginates, hydrocolloids, and films (Türsen, 2013). Furthermore, it is crucial to protect the environment from infectious agents. By incorporating antimicrobial properties into the wound dressings, the duration of treatment can be reduced. The first synthetic dressings, made from polyurethane film, were developed in the late 1960s, with additional forms emerging in the 1980s (Türsen, 2013).

According to the classification established by the U.S. Food and Drug Administration (FDA, 1999), wound dressings can be categorised into four types: i) non-resorbable passive dressings, such as gauze and tulle; ii) occlusive dressings, including film, hydrocolloid, and foam; iii) absorbent, hydrophilic dressings, such as alginates and hydrofibers; and iv) hydrogel dressings. These dressings can be enhanced through the incorporation of antimicrobial agents, growth factors, live cell cultures, keratin, collagen, and other substances to create bioengineered products (Dumville, 2012; Türsen, 2013). Commonly used antimicrobial agents include chlorhexidine acetate, silver, bismuth, and iodine. Additionally, two types of medical honey recommended by the FDA for direct application in wound care may also be utilized. In immunocompromised individuals, the use of natural antimicrobial agents in dressings is considered more beneficial than chemical agents due to their ability to reduce the risk of secondary systemic infections, their natural composition, patient comfort, sustainability, and lower cost. The chosen antimicrobial agent must be non-toxic to patients, should not induce antimicrobial resistance in the bacteria at the site of application, and must be effective against infectious agents (Nychas *et al*., 2003; Koire *et al*., 2024; Tüfekyapan *et al*., 2025). Among these natural chemical agents, thyme oil (*Thymus vulgaris* L.) from the *Lamiaceae* family exhibits antimicrobial activity through its active compounds, thymol and carvacrol, which affect cell permeability and disrupt membrane integrity (Borugă *et al*., 2014). Another natural oil, tea tree oil (from the *Myrtaceae* family, *Melaleuca alternifolia*), possesses properties due to its monoterpenoid components, which compromise pathogen cell membrane integrity and inhibit membrane-bound enzymes, rendering the microorganisms ineffective (Andrew *et al*., 1980; Uribe *et al*., 1985; Sikkema *et al*., 1995). Cinnamon oil, derived from *Cinnamomum zeylanicum* (*Lauraceae* family), exhibits antimicrobial effects and demonstrates effects against various microorganisms due to its components, including pyrimidine analogs, echinocandins, triazoles, eugenol, and cinnamaldehyde (Raharivelomanana, 1989; Mishra, 2008; Lopez, 2005). Cinnamaldehyde, an electronegative molecule found in cinnamon, exerts its antimicrobial effect by reacting with nitrogen-containing biomolecules such as proteins and nucleic acids. Furthermore, it has been reported that cinnamon extracts and oils inhibit bacteria by disrupting bacterial growth cell membranes, ATPases, and exhibiting anti-quorum sensing effects (Vasconcelos, 2018).

The disadvantages of chemical agents applied to wound dressings, such as skin discolouration, irritation, induction of bacterial resistance and cytotoxic effects on host tissues, have led to the use of natural products. Due to the need for biocompatible alternatives to traditional chemical agents in wound healing applications, we aimed to

evaluate the antimicrobial efficacy of essential aromatic oils by direct application to non-woven, hypoallergenic, flexible and adhesive, sterile and additive-free film occlusive wound dressings and non-resorbable gauze dressings. In our study, the inhibitory and microbicidal effects of thyme, tea tree and cinnamon oils were determined under *in vitro* conditions, and the antimicrobial activity of the essential oils on the gauze and hydrogel dressings was evaluated by adapting the standard methods used to evaluate the antimicrobial activity of textile products.

Materials and Methods

Preparation of the test microorganisms

In this study, the bacteria *Staphylococcus aureus* ATCC 6538 and *Escherichia coli* ATCC 8739, along with the yeast *Candida albicans* ATCC 10231, were used. *Melaleuca alternifolia* (tea tree), *Thymus vulgaris* L. (thyme oil), *Cinnamomum cassia* (cinnamon oil), and commercially available wound care dressings (non-antimicrobial) were utilised.

The microbial strains used in the experiments were stored at −86°C in a phosphate buffer and glycerol suspension. For experimental purposes, the frozen bacteria were revived by inoculating them onto Nutrient Agar (NA, Oxoid) and incubating at 37°C for 24 h. The cultured bacteria were then diluted in 1/500 Nutrient Broth (NB, Oxoid) to achieve a final concentration of 1-2×10^5 colony-forming units (CFU)/mL, starting from an initial density of 1-2×10^8 CFU/mL, as measured by a densitometer in sterile phosphate buffer (BioSan, Letonia).

Candida albicans was revived by inoculating Tryptic Soy Agar (TSA, Oxoid) and incubating at 30°C for 24 to 48 h. After the following incubation period, a turbidimetric suspension was prepared in Tryptic Soy Broth (TSB, Oxoid) and Sabouraud Dextrose Broth (SDB, Oxoid) to achieve a final concentration of 10^7 CFU/mL.

Evaluation of the Antimicrobial Activity of Tested Essential Oils Under in vitro Conditions

The antimicrobial activity of the selected essential oils in the suspension form was initially determined using the microdilution method. Subsequently, the active oils were applied to the wound dressings, and their antibacterial activities were assessed using standard testing methods.

Determination of the MIC and the MBC/MFC of the Tested Essential Oils

According to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2015; Aydoğdu *et al.*, 2023), microdilution tests were performed by adding 100 µl of bacterial suspension to wells containing different concentrations of the selected essential oils to obtain a final concentration of approximately 5×10^5 CFU/mL bacterial suspension in the wells. After an incubation period of 24 h at 37°C, the first well in which no bacterial growth was observed in the oil/bacterial suspension series was identified as the MIC. To determine the MBC and MFC values, 10 µl samples from the wells with no bacterial or fungal growth were plated onto Mueller Hinton Agar (MHA; Oxoid) Petri dishes and incubated at 37°C for an additional 24 h. The lowest concentration at which no colony formation occurred was recorded as the MBC/MFC dose. The MIC, MBC, and MFC tests were performed in triplicate alongside the positive and negative controls. A quaternary ammonium compound known to have antimicrobial activity against the test organisms was used as a positive control and sterile phosphate buffer used to dilute the bacteria was used as a negative control.

Determination of the Antimicrobial Activity of the Tested Oils Applied to Wound Dressing and Gauze

The antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans* was evaluated through a stepwise approach that included both qualitative and quantitative tests, using positive and negative controls as follows:

i) AATTC (Association of Textile, Appareal&Materials Professionals) Test Method 147 (Parallel Streak Method)

ii) EN ISO 20645 Determination of Antibacterial Activity - Agar Diffusion Plate Test

iii) AATCC 100 Test Method for the Determination of the Antibacterial Activity of Textile Materials

Assessment of the antimicrobial activity according to AATTC Test Method 147 (Parallel Streak Method)

The method used in this test was designed for the qualitative assessment of bacteriostatic, fungistatic, and antimicrobial activity in the samples.

Wound dressing samples, both with and without the application of an antimicrobial agent (control), were pre-

pared as rectangular pieces measuring 25×50 mm using sterile forceps, scissors, scalpels, and moulds.

The suspensions of the test microorganisms were prepared separately by adding 1.0±0.1 mL of a 24h liquid culture to 9.0±0.1 mL of sterile phosphate buffer in sterile test tubes. For inoculation, one drop of the test microorganism's suspension, obtained using a 4 mm diameter inoculating loop, was applied in five parallel lines on the surface of sterile agar plates. The lines were arranged to be approximately 60 mm long, with 10 mm spacing between them, and centred in the Petri dish. No additional sample collection was performed using the loop during the line inoculation, and care was taken to avoid any damage or tearing of the agar surface.

The sterile samples, both with and without the application of antimicrobial agents, were positioned perpendicular to the five inoculation lines to ensure complete contact between the samples and the bacterial lines. To prevent the samples from curling during incubation and from losing contact with the test microorganisms, sterile glass covers were placed over them. The Petri dishes were incubated at 37±2°C for 18-24 hours. At the end of the incubation period, the Petri plates were assessed for the presence or absence of a zone of inhibition, specifically noting any interruptions in the bacterial inoculum lines and clear zones of growth inhibition around the edges of the samples.

The inhibition zone along the inoculum line on one side of the tested sample was calculated using the following formula:

$W = \frac{T-D}{2}$, where: W=Inhibition zone, in mm; T=Total of the sample and inhibition zone, in mm; D=The test sample, in mm.

According to the AATCC 147 standard, the size of the inhibition zone cannot be used for the quantitative evaluation of antimicrobial activity. The absence of microorganism colonies in the contact area under the sample is regarded as acceptable antimicrobial activity (AATCC 147, 2004; Kimiran Erdem & Sanli Yurudu, 2008).

Assessment of the antimicrobial activity according to EN ISO 20645

A semi-quantitative test method (EN ISO 20645:2004) was employed to validate the antimicrobial activity of the samples. Antimicrobial oil-treated and untreated control samples were prepared in a circular shape with a diameter of 25±5 mm using sterile forceps, scissors, a scalpel, and moulds. Since the samples were tested between two layers of agar, both sides could be evaluated. The lower layer was free of bacteria, while the upper layer was inoculated with the selected microorganism.

The Petri dish and its sterility were checked. For the upper layer, 1 mL of the microorganism suspension (1×10^8 CFU/mL) was added to the melted and cooled nutrient agar (at 45°C). The selected microorganism was inoculated into this upper layer. The test samples were placed in the lower layer using sterile forceps, and 5 mL of agar containing the test microorganism was poured over the samples. After the agar solidified, the Petri dishes were incubated at 37±2°C for 18-24 hours.

Non-sterile samples, both with and without the application of antimicrobial agents, were evaluated in parallel in triplicate.

At the end of the incubation period, the Petri plates were evaluated for the presence or absence of a zone of inhibition surrounding the edges of the sample, as well as any growth beneath the sample.

The inhibition zone surrounding the tested sample was calculated using the following formula:

$(H =)\frac{(D-d)}{(2)}$, where: H=Inhibition zone, in mm; D=Total of the sample and inhibition zone, in mm; d=Diameter of the test sample, in mm.

After measuring the inhibition zone, the samples were carefully removed using sterile forceps, and the presence or absence of growth beneath each sample was assessed. The evaluation was conducted in accordance with Table 1.

Table 1

Evaluation of the zone of inhibition according to the EN ISO 20645 standard.

Inhibition Zone (mm)	Growth[a]	Evaluation
> 1	None	Good Effect
1-0	None	
0	None	
0	Weak	Limited Effect
0	Moderate	Ineffective
0	Intense	

[a]: Growth under the sample

Measurement of Antimicrobial Efficacy in accordance with the AATCC 100 Test Method

The reduction in the microorganism count in the samples was assessed using a quantitative testing method based on the AATCC 100 standard (AATCC 100, 2019).

Both antimicrobial oil-treated and untreated control samples were prepared in a circular shape with a diameter of 4.8±0.1 cm using sterile forceps, scissors, scalpels, and moulds. Before testing, the number of samples capable of absorbing 1 mL of inoculum was determined. It was found that 1 mL of the bacterial suspension could be absorbed by one pair of double-layered gauze and a single-layer wound dressing sample. The samples were then impregnated with 1 mL of bacterial suspension at a concentration of 1-2×10^7 CFU/mL, which was added to separate sterile containers with screw caps.

After a contact time of 24 h, a neutralising solution was added to the wound dressing and gauze samples that had absorbed the test inoculum to stop the activity of the antimicrobial compounds. A neutralising solution consisting of 3% Tween 80 and 0.3% lecithin in a liquid medium was used. At the end of each contact period, the bottles were vortexed, and serial dilutions were performed. From each dilution tube, 0.1 mL was taken and spread onto Petri dishes containing nutrient agar, which were then incubated at 37°C for 18-24 hours. After the incubation period, the colonies that formed were counted.

The reduction in colony counts was calculated using the following formula:

$R = \frac{(C-A)}{(C)}(\times 100)$, where: R=Percentage reduction; A=Colony count obtained from antimicrobial oil-treated samples after 24 h of contact; C=Colony count obtained from untreated samples at time 0 (approximately 1 minute after measurement).

According to the AATCC 100 standard, the acceptable kill rate percentage's lower and upper limits are established by the involved parties. Additionally, the standard indicates that a deviation of up to 18% may occur between different analytical laboratories, while a variation of up to 8% can be observed within analyses conducted by the same individual.

Statistical analysis

Comparisons of differences in CFU between the control group and those treated with essential oils were analysed using Student's t-test. $P < .05$ was considered statistically significant.

Results

Determination of the MIC and the MBC/MFC of the Tested Essential Oils Against the Test Microorganisms

The MIC and MBC/MFC of the tested essential oils against the selected test microorganisms are demonstrated in Table 2. The MIC/MBC/MFC results revealed that the essential oils exhibited varying sensitivities against the tested microbial strains. As indicated in Figure 1 and Table 1, the antimicrobial activities of the oils were positively correlated with their concentrations for the tested bacterial strains.

The most effective inhibition was observed against *C. albicans*, followed by *E. coli* and *S. aureus* (Table 2). The lowest MIC and MBC concentrations were determined at a concentration of 0.25 mg/mL for thyme oil against *S. aureus* and *E. coli* and cinnamon oil against *E. coli*.

Table 2

The MIC and the MBC/MFC values of the tested essential oils against test microorganisms (mg/mL).

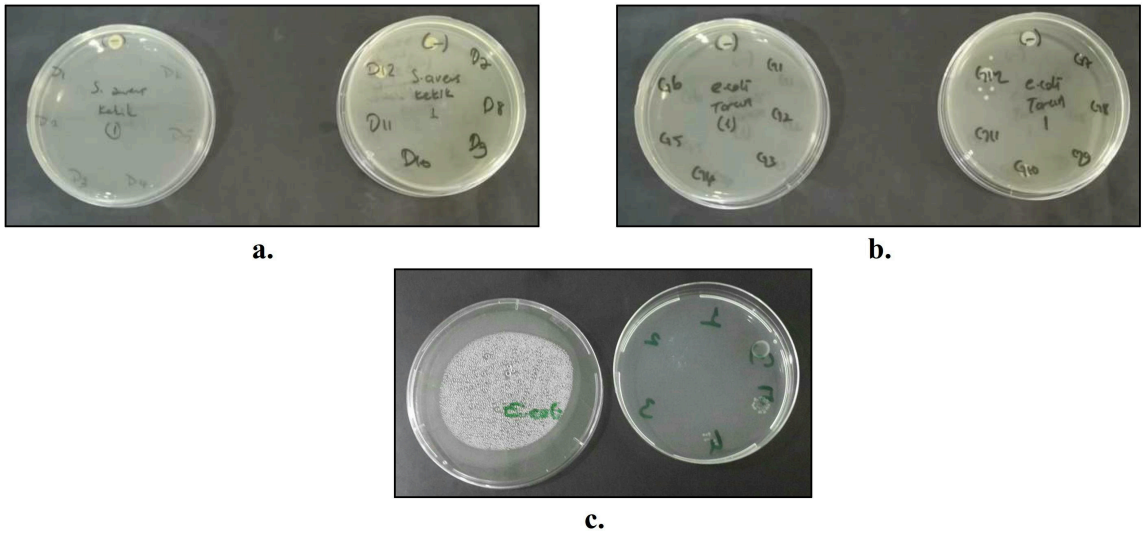
Test microorganisms	Oils	MIC	MBC
S. aureus	Tea Tree Oil	1	3
	Thyme Oil	0.25	0.25
	Cinnamon Oil	0.625	0.625
E. coli	Tea Tree Oil	1	2
	Thyme Oil	0.25	0.25
	Cinnamon Oil	0.25	0.25
C. albicans	Tea Tree Oil	1	2
	Thyme Oil	0.31	0.31
	Cinnamon Oil	0.31	0.31

Determination of the Antimicrobial Activity of the Tested Oils Applied to Wound Dressing and Gauze

The antimicrobial activity of the wound dressing and gauze samples impregnated with essential oils was evaluated using qualitative (AATCC 147), semi-quantitative (EN ISO 20645) and quantitative (AATCC 100) methods.

Figure 1

Representative photographs of the determination of the MBC values for the tested oils; a) thyme oil against S. aureus, b) cinnamon oil against E. coli, c) tea tree oil against E. coli.



a. b. c.

Assessment of the antimicrobial activity according to AATTC Test Method 147 (Parallel Streak Method)

The qualitative AATCC 147 test method results showing the bacteriostatic effect are shown in Tables 3, 4, and 5.

As shown in Figures 2 and 3, according to the results of the AATCC 147 test, all oils evaluated, except tea tree oil, formed inhibition zones against representative pathogens. In accordance with the AATCC 147 standard, the absence of growth in the contact area of the antimicrobial-treated samples was considered to have a good antimicrobial effect (Figures 2 and 3; Tables 3, 4 and 5).

Table 3

The results of the antimicrobial activity of essential oil-impregnated wound dressings and gauze against S. aureus ATCC 6538 using the parallel streak method.

Sample	T[1]	T-D[1]	(T-D/2)[1]	Growth
	WD/G	WD/G	WD/G	WD/G
Tea Tree Oil	-/-	-/-	-/-	No/No
	-/-	-/-	-/-	No/No
	-/-	-/-	-/-	No/No
	43.50/47.00	18.50/22.00	9.25/11.00	No/No
	45.00/53.00	20.00/28.00	10.00/14.00	No/No
Thyme Oil	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
Cinnamon Oil	37.50/37.50	12.50/12.50	6.25/6.25	No/No
	42.50/45.00	20.00/20.00	10.00/10.00	No/No
	42.50/54.00	27.00/27.00	13.50/13.50	No/No
	42.50/56.50	24.50/24.50	15.75/15.75	No/No
	42.50/58.50	33.50/33.50	16.75/16.75	No/No

[1]mm, D = 25 mm, WD: wound dressing, G: gauze. Growth was vigorous around the control samples, and no inhibition of growth was observed in their vicinity.

Figure 2

Representative photographs of the results of the antimicrobial activity of the essential oil-impregnated wound dressing and gauze against S. aureus ATCC 6538 using the parallel streak method.

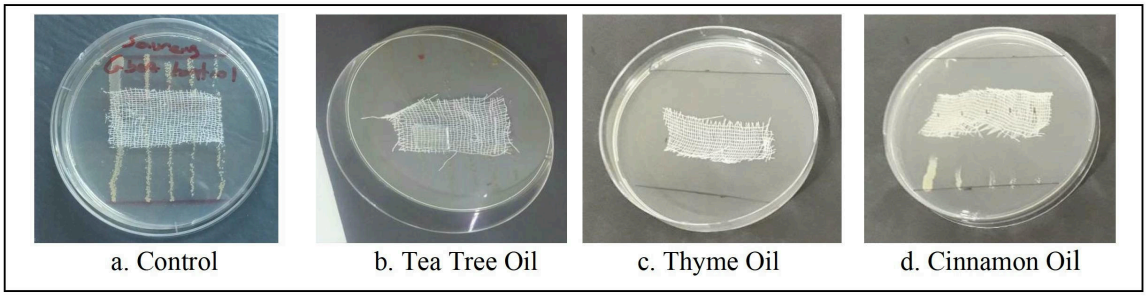


Figure 3

Representative photographs of the results of the antimicrobial activity of the essential oil-impregnated wound dressing and gauze against E. coli ATCC 25922 using the parallel streak method.

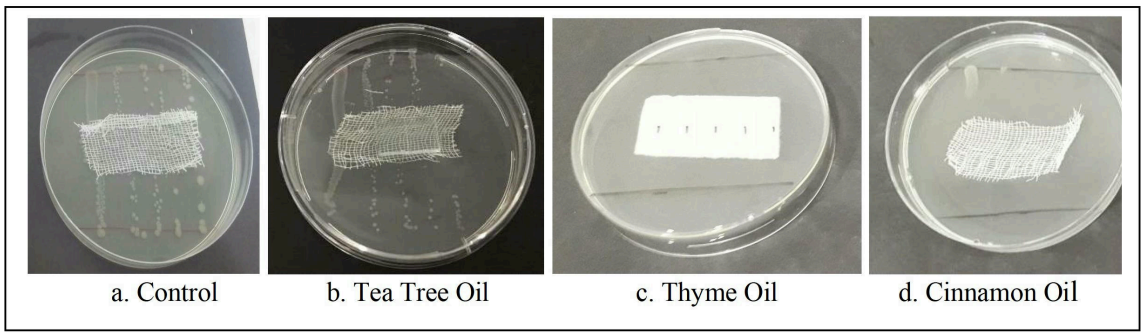


Table 4

The results of the antimicrobial activity of the essential oil-impregnated wound dressings and gauze against E. coli ATCC 25922 using the parallel streak method.

Sample	T[1]	T-D[1]	(T-D/2)[1]	Growth
	WD/G	WD/G	WD/G	WD/G
Tea Tree Oil	-/-	-/-	-/-	No/No
	-/-	-/-	-/-	No/No
	-/-	-/-	-/-	No/No
	-/-	-/-	-/-	No/No
	45.00/46.00	20.00/21.00	10.00/10.50	No/No
Thyme Oil	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
Cinnamon Oil	37.50/37.50	12.50/12.50	6.25/6.25	No/No
	58.00/58.00	33.00/33.00	16.50/16.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No

[1]mm, D = 25 mm, WD: wound dressing, G: gauze. Growth was vigorous around the control samples, and no inhibition of growth was observed in their vicinity.

Table 5

The results of the antimicrobial activity of essential oil-impregnated wound dressings and gauze against C. albicans ATCC 10231 using the parallel streak method.

Sample	T[1]	T-D[1]	(T-D/2)[1]	Growth
	WD/G	WD/G	WD/G	WD/G
Tea Tree Oil	-/-	-/-	-/-	No/No
	-/-	-/-	-/-	No/No
	-/-	-/-	-/-	No/No
	-/-	-/-	-/-	No/No
	51.00/52.00	26.00/27.00	13.00/13.50	No/No
Thyme Oil	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
Cinnamon Oil	34.50/37.50	12.50/12.50	6.25/6.25	No/No
	42.50/44.00	17.50/19.00	8.75/9.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No
	60.00/60.00	35.00/35.00	17.50/17.50	No/No

[1]mm, D = 25 mm, WD: wound dressing, G: gauze. Growth was vigorous around the control samples, and no inhibition of growth was observed in their vicinity.

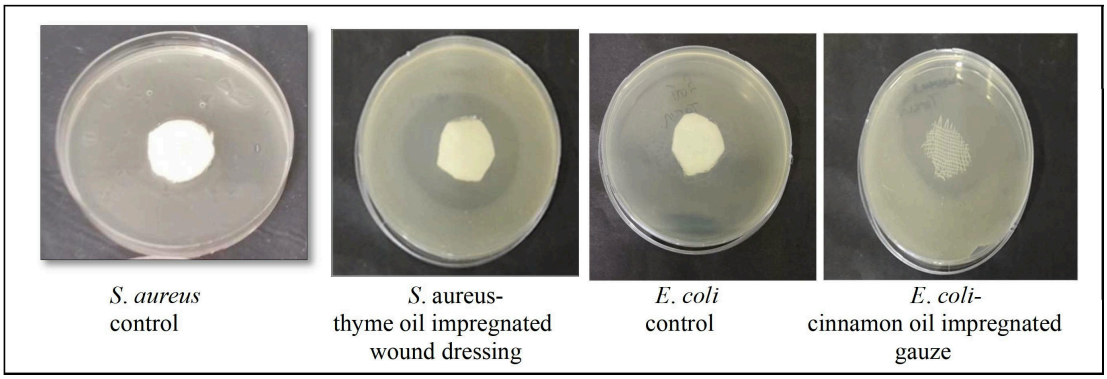
Table 6

The results of the antimicrobial activity of wound dressings and gauze impregnated with essential oils according to the standard test ISO 20645.

	Samples	*S. aureus* ATCC 6538	*E. coli* ATCC 25922	*C. albicans* ATCC 10231
Tea Tree Oil	Wound Dressing	0 mm and no growth under the sample	0 mm and no growth under the sample	0 mm and no growth under the sample
	Gauze	0 mm and no growth under the sample	0 mm and no growth under the sample	0 mm and no growth under the sample
	Negative Control	0 mm and intense growth	0 mm and intense growth	0 mm and intense growth
Thyme Oil	Wound Dressing	30.8 mm and no growth under the sample	33.2 mm and no growth under the sample	42.5 mm and no growth under the sample
	Gauze	35 mm and no growth under the sample	33 mm and no growth under the sample	42.5 mm and no growth under the sample
	Negative Control	0 mm and intense growth	0 mm and intense growth	0 mm and intense growth
Cinnamon Oil	Wound Dressing	14 mm and no growth under the sample	21,2 mm and no growth under the sample	42.5 mm and no growth under the sample
	Gauze	50 mm and no growth under the product	17 mm and no growth under the sample	42.5 mm and no growth under the sample
	Negative Control	0 mm and intense growth	0 mm and intense growth	0 mm and intense growth

Figure 4

Representative photographs of the results of the antimicrobial activity of the essential oil-impregnated wound dressing and gauze using the ISO 20645 standard method.



EN ISO 20645 (Determination of Antibacterial Activity-Agar Diffusion Plate Test)

The results and representative photographs of the oil-impregnated wound care samples according to the EN ISO 20645 standard method are shown in Table 6 and Figure 4 (Table 6, Figure 4).

In accordance with the provisions stipulated in the EN ISO 20645 standard, the efficacy of the essential oil-impregnated wound care samples was demonstrated to be effective against the bacteria under investigation. This was evidenced by the absence of bacterial growth beneath the samples (Tables 3, 5, and Figure 4).

Measurement of Antimicrobial Efficacy in accordance with the AATCC 100 Test Method

The results of the evaluation of the antimicrobial activity of the essential oil-impregnated wound care products according to the AATCC 100 method are shown in Table 7.

Wound care samples impregnated with essential oils showed strong antimicrobial activity with a reduction in test microorganisms of more than 4 log, 99.99% (Table 7).

Discussion

Since ancient times, people have utilised linen, cotton, and gauze to dress wounds, along with various natural herbal products, including aromatic oils, to prevent infections (Simões *et al.*, 2018). Many scientific studies have shown that extracts obtained from plants' roots, leaves, or flowers can have a therapeutic effect due to compounds such as alkaloids, flavonoids, glycosides, and terpenes (Akpınar *et al.*, 2024; Türsen, 2013). Extracts can increase collagen synthesis, stimulate fibroblasts, support epithelization, and provide antioxidant and antimicrobial effects (Toroğlu & Çenet, 2013). Among the herbal products studied, aromatic oils are primarily preferred due to their advantages, such as being non-toxic to tissues, easily accessible, and cost-effective (Seow *et al.*, 2014). Treatments that were once developed through trial and error are now validated through standardised antimicrobial activity and cytotoxicity tests conducted in laboratories. These advancements provide a range of contemporary products with applications in bioengineering.

While it was once preferred to keep wounds dry, research conducted over the past 50 years has demonstrated that a moist environment facilitates the clearance of debris and the elimination of bacteria. This is attributed to the presence of fluids and electrolytes as well as epithelial growth factors, matrix metalloproteinase enzymes, macrophages, and neutrophils. In addition to protecting against contamination, enhancing patient comfort, and facilitating wound monitoring through their transparency, wound dressings can serve multiple purposes by incorporating unique properties, such as an antimicrobial effect achieved through various chemical compounds (Thiruvoth, 2015; Türsen, 2013).

Chemical compounds often preferred in wound dressings include silver, silver products, polyhexamethylene biguanide, cadoxemer iodine or polyacrylate used to reduce the risk of superficial infection in chronic wounds. Of these, silver in particular is effective against a wide range of resistant microorganisms, but it has been found to have a negative effect on *in vitro* skin cultures, and its effect on healthy tissue is unknown. Chlorhexidine, betadine, acetic acid, hydrogen peroxide, scarlet red dye and bacitracin are used to prevent superficial infections. Antimicrobials are known to have local effects such as pain and rash and

Table 7

Results of the antimicrobial activity of tea tree oil-impregnated wound care products using the AATCC 100 method.

Microorganisms	Materials	Bacteria count**	% Percentage of reduction
	WD-tea tree oil	< 100**	> 99.99978
	WD-thyme oil	< 100**	> 99.99978
	WD-cinnamon oil	< 100**	> 99.99978
***S. aureus* ATCC 6538**	G-tea tree oil	< 100**	> 99.99978
	G- thyme oil		> 99.99978
	G- cinnamon oil	< 100**	> 99.99978
	Control	$2.55x10^5$ (0.h)	$4.60x10^7$ (24.h)
	WD-tea tree oil	< 100**	> 99.9988
	WD-thyme oil	< 100**	> 99.9988
	WD-cinnamon oil	< 100**	> 99.9988
***E. coli* ATCC 25922**	G-tea tree oil	< 100**	> 99.9988
	G- thyme oil	< 100**	> 99.9988
	G- cinnamon oil	< 100**	> 99.9988
	Control	$2.14x10^5$ (0.h)	$8.90 x10^6$
	WD-tea tree oil	< 100**	> 99.9962
	WD-thyme oil	< 100**	> 99.9962
	WD-cinnamon oil	< 100**	> 99.9962
***C. albicans* ATCC 10231**	G-tea tree oil	< 100**	> 99.9962
	G- thyme oil	< 100**	> 99.9962
	G- cinnamon oil	< 100**	> 99.9962
	Control	$2.44x10^5$ (0.h)	$2.70 x10^6$

**Bacteria counts after 24 h of exposure

**A colony count of 0 in the Petri dish was accepted as a 100 colony count due to the dilution factor.

cytotoxic effects on cells necessary for the wound healing process such as epithelial cells, fibroblasts, endothelial and inflammatory cells and others; systemic effects such as causing toxicity in the kidneys, liver and other organs as a result of absorption of these substances into the systemic circulation. It is well known that iodine and silver compounds cause skin discolouration and irritation with prolonged use; antiseptics have a broader spectrum and higher cytotoxicity in host tissues than antibiotics, while antibiotics are more likely to develop bacterial resistance (Borda *et al*., 2016; Punjataewakupt *et al*., 2019). Therefore, considering the biocompatibility of natural products against the disadvantages of chemicals, natural products are preferred. In our study, taking all these factors into account, the essential aromatic oils tested were applied directly to non-woven, hypoallergenic, flexible and adhesive, sterile and additive-free film occlusive wound dressings and non-resorbable gauze. The dressings tested in the study were preferred because they did not contain any interfering substances.

Choosing the optimal concentration of antimicrobial is important both to adequately control infection and to avoid toxicity from antimicrobial administration. The concentration of antimicrobial required to inhibit the growth of a pathogen was determined with MIC and MBC tests. According to our MIC and MBC test results, the highest MIC/MBC (MFC) concentrations (1-3 mg/mL) were obtained with tea tree oil; the lowest MIC and MBC concentrations were obtained at a concentration of 0.25 mg/mL for thyme oil against *S. aureus* and *E. coli* bacteria and for cinnamon oil against *E. coli* bacteria. Tea tree oil and cinnamon oil were effective against the fungus *C. albicans* at lower concentrations than bacteria. The MIC and MBC values obtained for oils are consistent with the results evaluated for similar microorganisms (Costa *et al*., 2021; Gao *et al*., 2020; Shi *et al*., 2016; Yasin *et al*., 2021).

Following the application of the MBC doses determined in our study to the wound dressing and gauze, the samples were evaluated using a qualitative to quantitative exper-

imental set-up with a stepwise approach to assess the static and cidal effects. Qualitative and semi-quantitative tests showed that all essential oils had good antimicrobial activity, including those that did not form a zone (tea tree oil), and this effect was confirmed by the standard qualitative method. The wound dressing samples impregnated with essential oils were found to have strong antimicrobial activity with a 99.99% reduction of more than 4 logs of test microorganisms by qualitative assessment. The results of the present study indicate that the essential oils tested have significant ($p<0.05$) antimicrobial activity, strengthening the potential use of these substances as antimicrobials in the future.

It is known that in various studies in the literature, one or two of these oils have been applied with chemical agents that may have stabilising and/or synergistic effects on the wound dressing and evaluated using various standard antimicrobial activity tests (Behary *et al*., 2020; Besen, 2019; Cremar *et al*., 2018; Gheorghita *et al*., 2022; Rieger *et al*., 2014). In our study, unlike other studies, three different essential oils were applied to sterile gauze and wound dressing without any other additive and their antimicrobial activity was evaluated using a stepwise qualitative to quantitative experimental design. This study has demonstrated the potential of natural essential oils to provide an effective solution by demonstrating an antimicrobial effect when applied to wound dressing material through *in vitro* treated dressing tests.

Conclusion

In conclusion, based on the results of our study, wound dressings with natural essential oils applied to reduce the risk of infection and indirectly promote wound healing can reduce healing time and improve patient quality of life in a cost-effective care setting.

Ethics Approval: No specific ethical approval was necessary, and no ethical contraventions occurred in this report.

Peer Review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- N.Ö.Ş.; Data Acquisition- N.Ö.Ş., B.K.; Data Analysis/Interpretation- N.Ö.Ş., B.K.; Drafting Manuscript- N.Ö.Ş., B.K.; Critical Revision of Manuscript- N.Ö.Ş.; Final Approval and Accountability- N.Ö.Ş.; Technical or Material Support- N.Ö.Ş., B.K.; Supervision- N.Ö.Ş.

Acknowledgements: This study was carried out by Beyza Karakus under the supervision of Nazmiye Ozlem Sanli as a graduate thesis of IU Faculty of Science, Higher Education Council (YÖK) Basic Sciences Programmes (TEBIP).

Conflict of Interest: The authors have no conflict of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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