



Unveiling the antimicrobial potential of essential oils from medicinal and aromatic plants against foodborne pathogens

Aromatik ve tıbbi bitkilerden elde edilen esansiyel yağların gıda kaynaklı patojenlere karşı antimikrobiyal potansiyelinin ortaya çıkarılması

Seda Altuntas^{1*} , Ayca Yigit Cinar¹ , Oya Kacar² , Sema Demir³

¹Department of Food Engineering, Faculty of Engineering and Natural Sciences, Bursa Technical University, Bursa, Türkiye.
seda.altuntas@btu.edu.tr, ayca.yigit@btu.edu.tr

²Department of Field Crops, Faculty of Agriculture, Bursa Uludağ University, Bursa, Türkiye.
okacar@uludag.edu.tr

³Central Research Institute of Food and Feed Control, Bursa, Türkiye.
sema.demir@tarimorman.gov.tr

Received/Geliş Tarihi: 16.05.2025
Accepted/Kabul Tarihi: 13.12.2025

Revision/Düzeltilme Tarihi: 01.12.2025

doi: 10.65206/pajes.84738

Research Article/Araştırma Makalesi

Abstract

This study investigates the chemical composition and antimicrobial properties of essential oils (EOs) from rosemary (*Rosmarinus officinalis*), sage (*Salvia officinalis*), basil (*Ocimum basilicum*), peppermint (*Mentha piperita*), and spearmint (*Mentha spicata*). Oxygenated monoterpenes were identified as the dominant compound class across all species, with the highest proportions in *Mentha piperita* (90.7%) and *Mentha spicata* (78.1%). Among individual compounds, 1,8-cineole was prominent, particularly in rosemary (29.7%) and sage (23.6%). Unique profiles were observed for linalool in basil (53.2%) and menthol in peppermint (50%), while carvone dominated in spearmint (65%). Antimicrobial assays showed that *Mentha piperita* exhibited the strongest activity, particularly against *L. monocytogenes* ATCC 7644, with a minimum inhibitory concentration (MIC) of 16 µg/mL. This activity is attributed to its high menthol content. In contrast, *Rosmarinus officinalis* and *Salvia officinalis* exhibited limited or no activity against certain bacterial strains. *E. coli* O157:H7 NTCC12900 was identified as the most sensitive bacteria to the tested EOs, particularly peppermint and sage oils. These results highlight the promise of essential oils as natural antimicrobial agents. Their efficacy against foodborne pathogens suggests promising applications in food preservation. Incorporating EOs into packaging materials or directly into food products offers an innovative approach to extending shelf-life and ensuring safety. Such strategies align with the growing demand for sustainable, chemical-free preservation techniques.

Keywords: Antimicrobial activity, Essential oils, Food pathogens, Chemical composition.

Öz

Bu çalışma, biberiye (*Rosmarinus officinalis*), adaçayı (*Salvia officinalis*), fesleğen (*Ocimum basilicum*), tıbbi nane (*Mentha piperita*) ve bahçe nanesi (*Mentha spicata*) bitkilerine ait uçucu yağların (essential oils, EOs) kimyasal bileşimini ve antimikrobiyal özelliklerini araştırmıştır. Tüm türlerde oksijenli monoterpenler baskın bileşik sınıfı olarak belirlenmiş olup, en yüksek oranlar *Mentha piperita* (%90.7) ve *Mentha spicata* (%78.1) türlerinde tespit edilmiştir. Bireysel bileşikler arasında ise 1,8-sineol özellikle biberiye (%29.7) ve adaçayında (%23.6) öne çıkmıştır. Fesleğende linalool (%53.2) ve nane uçucu yağında mentol (%50) yüksek oranda bulunurken, bahçe nanesi türünde ise karvon (%65) baskın bileşik olmuştur. Yapılan antimikrobiyal testlerde *Mentha piperita* en güçlü antimikrobiyal aktiviteyi göstermiş ve özellikle *Listeria monocytogenes* ATCC 7644 suşuna karşı minimum inhibitör konsantrasyonu (MIC) 16 µg/mL olarak belirlenmiştir. Bu yüksek aktivite, mentol içeriğinin yüksekliği ile ilişkilendirilmiştir. Buna karşılık, *Rosmarinus officinalis* ve *Salvia officinalis* bazı bakteri suşlarına karşı sınırlı ya da hiç aktivite göstermemiştir. Çalışmada test edilen uçucu yağlara karşı en duyarlı bakteri olarak *E. coli* O157:H7 NTCC12900 suşunun olduğu belirlenmiş; özellikle nane ve adaçayı yağlarına karşı yüksek duyarlılık göstermiştir. Bu bulgular, uçucu yağların doğal antimikrobiyal ajanlar olarak potansiyelini vurgulamakta olup, gıda kaynaklı patojenlere karşı etkinlikleri, gıda koruma uygulamalarında umut verici alternatifler olduğunu göstermektedir. Uçucu yağların ambalaj materyallerine veya doğrudan gıda ürünlerine eklenmesi, raf ömrünü uzatmak ve gıda güvenliğini sağlamak amacıyla yenilikçi bir yaklaşım sunmakta ve sürdürülebilir, kimyasal içermeyen koruma tekniklerine yönelik artan talep ile örtüşmektedir.

Anahtar kelimeler: Antimikrobiyal aktivite, Uçucu yağlar, Gıda patojenleri, Kimyasal bileşim.

1 Introduction

For centuries, plants and their extracts have served as important resources in managing numerous diseases owing to their therapeutic effects. Additionally, these plants are valued in areas such as cosmetics, the food industry, aromatherapy, and phytotherapy because of their aromatic characteristics. Essential oils (EOs) derived from medicinal plants are volatile, aromatic, and complex compounds obtained through methods such as extraction, pressing, or steam distillation from different

plant parts like flowers, leaves, and seeds [1],[2]. The antimicrobial activity of EOs varies depending on the diversity, concentration, and type of plant constituents. Most EOs have been granted GRAS (Generally Recognized as Safe) designation, indicating their safety and non-toxic nature. Furthermore, studies have reported that EOs exhibit significant antimicrobial effects, demonstrating inhibitory activity against pathogen bacteria as well as yeasts [3]. These antimicrobial properties are mainly attributed to their bioactive compounds such as phenolic compounds, terpenes, and aldehydes which can

*Corresponding author/Yazışılan Yazar

interact with the lipid bilayer of the cell membrane, leading to increased permeability and leakage of cellular contents. Moreover, these compounds may alter membrane potential, denature proteins, and interfere with enzyme activity involved in essential metabolic processes. Such multifaceted mechanisms ultimately result in the inhibition of microbial growth or cell death [4].

Numerous pathogenic bacteria associated with foodborne diseases exist, among which the most significant and critical pathogens include, *Salmonella* spp., *Campylobacter* spp., *Clostridium* spp., *Shigella* spp., *Vibrio* spp., *Yersinia enterocolitica*, *Brucella* spp., and *Aeromonas* spp. [5]. The increasing prevalence of antibiotic-resistant bacteria has raised global concerns among health authorities, emphasizing the urgent need for alternative antimicrobial strategies. As a result, there is growing interest in naturally derived antimicrobial compounds, including essential oils, due to their safety, bioactivity, and versatility. EOs have demonstrated potential not only as therapeutic agents but also as natural preservatives in the food industry, components in pharmaceutical formulations, and active elements in antimicrobial packaging. Their application across these sectors offers a promising approach to mitigate microbial contamination and reduce reliance on synthetic antimicrobials, addressing both public health concerns and consumer demand for natural products.

Rosemary (*Rosmarinus officinalis* L.), an aromatic herb native to the Mediterranean and part of the *Lamiaceae* family, has gained attention for its antioxidant and antimicrobial effects. Its use in the food industry encompasses both flavor enhancement and preservation, contributing to prolonged shelf life [6]. Sage (*Salvia officinalis* L.), another *Lamiaceae* species, is utilized both as a tea and as a flavor enhancer, recognized for its functional properties [7]. Basil (*Ocimum basilicum* L.) is widely applied in food, cosmetics, and traditional medicine due to its EOs and has notable antimicrobial effects. Particularly, purple basil varieties are valued in the food industry as a natural colorant due to their anthocyanin content [8]. Peppermint (*Mentha piperita* L.) and spearmint (*Mentha spicata* L.) species, containing commercially valuable EOs, are primarily used for their menthol content in cosmetics, pharmaceuticals, and the food industry. *Mentha piperita*, a perennial plant native to Southern Europe, is the most widely used mint species worldwide [9]. In contrast, *Mentha spicata*, which has a distinct aroma profile due to its high carvone content, stands out from other mint species [3].

This study evaluates the antimicrobial effects of EOs obtained from rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.), basil (*Ocimum basilicum* L.), and two mint species (*Mentha piperita* L. and *Mentha spicata* L.) against major foodborne pathogens, including *Staphylococcus aureus* ATCC 29213, *Escherichia coli* O157:H7 NTCC12900, and *Listeria monocytogenes* ATCC 7644. Additionally, the chemical compositions of these EOs were analyzed by GC-MS. The findings of this study will contribute to a better understanding of the relationship between EO composition and antimicrobial efficacy, providing insights for potential applications such as natural food preservatives and alternative antimicrobial agents.

2 Materials and methods

2.1 Plant material and preparation of EOs

The plant material used in this study was obtained from plantations at the Medicinal and Aromatic Plants experimental

field in the Department of Field Crops, Faculty of Agriculture, Uludağ University, during the 2022 vegetation period. The harvested plant materials were air-dried in the shade under room conditions, and then the leaves and stems were separated. EOs were extracted from leaves using a Neo-Clevenger Apparatus (Light Essential Oil Determination Device) by the hydrodistillation method, which enables volumetric measurement of volatile oils. For this purpose, 20 g of dried leaves was macerated for a brief period with 200 mL of water in a ground glass flask before being placed in the distillation apparatus, where the distillation process continued for three hours. The percentage of EO was determined by reading the measurement from the burette section of the apparatus. The extracted EOs were stored at +4 °C until analysis [10].

2.2 Gas chromatography

Gas chromatography-mass spectrometry (GC-MS) was used to determine the percentage compositions of essential oils obtained by hydrodistillation. Gas chromatography-mass spectrometry (GC-MS) analyses were performed with an Agilent 7890A GC and a 5975C MS, employing an HP Innowax capillary column (60 m × 0.25 mm × 0.25 µm). Before analysis, essential oil samples were diluted 1:100 in hexane (Merck KGaA, Darmstadt, Germany). Helium gas at a flow rate of 0.8 mL/min was used as the carrier, and 1 µL of each sample was injected with a split ratio of 40:1. The injector temperature was set to 250 °C, the column temperature program was set to 60 °C (10 minutes), from 60 °C to 220 °C at 4 °C/minute and 220 °C (10 minutes). Identifications of analytes were established by comparing the observed mass spectral data with the WILEY, ADAMS and NIST MS library database, with similarity (Sim) values presented in (typically ≥85% when compared to NIST/Wiley /ADAMS Libraries). Retention Index values were computed for all observed peaks to enhance the reliability of the identifications. Data for an alkane standard (C8-C20) was obtained and utilized for these conclusions. The recorded RI value is presented in Table 1 [11].

2.3 Preparation of bacterial cultures

The test strains of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* O157:H7 NTCC 12900, and *Listeria monocytogenes* ATCC 7644 were prepared using the direct colony suspension method. The bacterial strains were incubated in Nutrient Broth (NB) at 37 °C for 18-24 hours. Before antimicrobial analysis, the suspensions were standardized to a turbidity equivalent to the 0.5 McFarland standard [12].

2.4 Agar-well diffusion assay

Bacterial suspensions with an approximate density of 10⁷ CFU/mL were spread uniformly onto nutrient agar (NA) plates. After sufficient drying, wells were created in the agar (6 mm), into which 15 µL of the obtained essential oils (EOs) were transferred. Plates were incubated at 37 °C for 24 hours, and subsequently, inhibition zone diameters were recorded in millimeters [12]. Sterilized deionized water and the antibiotics (Gentamycin and Kanamycin) were added in this study as negative and positive control, respectively.

Table 1. The chemical profiles (%) of essential oils obtained from rosemary, sage, basil and two types of mints.

No	Compounds'	RI''	<i>Rosmarinus officinalis</i> L.	<i>Salvia officinalis</i> L.	<i>Ocimum basilicum</i> L.	<i>Mentha piperita</i> L.	<i>Mentha spicata</i> L.
1	α -Thujene	928	-	16.2±0.20	-	-	-
2	α -Pinene	936	-	-	-	-	-
3	Camphene	950	11.5±0.10 ^a	2.8±0.10 ^b	-	-	0.3±0.01 ^c
4	Sabinene	970	6.0±0.10 ^b	7.1±0.10 ^a	1.5±0.05 ^c	-	-
5	β -Pinene	978	0.2±0.01 ^b	-	0.2±0.01 ^b	0.3±0.01 ^b	1.0±0.05 ^a
6	Myrcene	989	1.3±0.01 ^b	4.8±0.10 ^a	0.95±0.01 ^c	0.4±0.01 ^d	1.6±0.05 ^b
7	α -Phellandrene	1004	2.5±0.01 ^a	-	1.3±0.01 ^b	-	0.5±0.01 ^c
8	Δ -3 carene	1011	0.3±0.01	-	-	0.3±0.01	-
9	α -Terpinene	1017	0.2±0.01 ^b	-	-	0.4±0.01 ^a	0.2±0.01 ^b
10	p-Cymene	1024	0.6±0.01 ^a	-	0.1±0.01 ^b	-	0.1±0.01 ^b
11	Limonene	1025	-	-	-	1.8±0.05	-
12	1,8 Cineole	1032	-	3.2±0.10 ^b	-	-	10.3±0.1 ^a
13	E- β -Ocimene	1048	29.7±0.50 ^a	23.6±0.20 ^b	12.4±0.20 ^c	6.4±0.10 ^e	10.8±0.20 ^d
14	γ -Terpinene	1060	-	-	0.4±0.01	-	-
15	Terpinolene	1087	0.8±0.02 ^a	-	-	-	0.3±0.01 ^b
16	Linalool	1099	0.3±0.01 ^a	-	0.1±0.01 ^b	-	-
17	Camphor	1143	4.8±0.10 ^b	-	53.2±0.30 ^a	-	-
18	Menthone	1151	19.8±0.20 ^a	19.5±0.20 ^a	1.1±0.02 ^b	-	0.2±0.01 ^c
19	Isoborneol	1158	-	-	-	28.0±0.30	-
20	Borneol	1166	0.9±0.01 ^c	3.5±0.02 ^a	1.0±0.01 ^b	-	-
21	Terpinene-4-ol	1177	9.3±0.20 ^a	3.9±0.10 ^b	-	-	-
22	Menthol	1177	-	0.2±0.01	-	-	1.1±0.01
23	Trans-carveol	1217	-	-	-	50.0±0.40	-
24	Pulegone	1234	0.1±0.01	-	-	-	-
25	Carvone	1242	-	-	-	0.3±0.01 ^a	0.4±0.01 ^b
26	Piperitone	1254	-	-	-	0.7±0.01 ^b	65±0.40 ^a
27	Neomenthyl acetate	1274	-	-	-	0.8±0.02	-
28	Bornyl acetate	1278	-	-	-	0.4±0.01	-
29	Isobornyl acetate	1286	1.1±0.02 ^b	2.0±0.02 ^a	0.7±0.01 ^c	-	-
30	Menthyl acetate	1296	-	-	0.3±0.01	-	-
31	Carvacrol	1300	-	-	-	3.9±0.05	-
32	Iso-menthyl acetate	1304	0.1±0.01 ^c	0.7±0.01 ^a	0.2±0.01 ^b	-	0.2±0.01 ^b
33	Trans-caryyl acetate	1336	-	-	-	0.2±0.01	-
34	α -Cubebene	1351	-	-	-	-	0.4±0.01
35	Eugenol	1358	-	-	0.2±0.01 ^b	-	0.7±0.02 ^a
36	α -Copaene	1376	-	-	11.3±0.20	-	-
37	β -Elemene	1390	-	-	0.3±0.01	-	-
38	β -Caryophyllene	1420	-	-	1.4±0.02	-	-
39	α -Humulene	1453	0.5±0.01 ^b	2.6±0.04 ^a	-	0.3±0.01 ^c	2.8±0.02 ^a
40	α -Farnesene	1500	0.2±0.01 ^b	-	0.4±0.03 ^a	-	-
41	Spathulenol	1576	0.2±0.01 ^b	-	2.7±0.05 ^a	-	-
42	Caryophyllene oxide	1581	-	-	0.5±0.01	-	-
43	α -Cadinol	1652	-	-	-	2.0±0.03 ^a	0.8±0.02 ^b
44	Grouped compounds		-	-	2.2±0.04 ^a	-	0.2±0.01 ^b
	Monoterpene hydrocarbons		23.7±0.10 ^b	34.1±0.10 ^a	4.55±0.10 ^d	3.2±0.05 ^e	14.3±0.10 ^c
	Oxygenated monoterpenes		65.8±0.10 ^d	53.4±0.10 ^e	80.2±0.50 ^b	90.7±0.60 ^a	78.1±0.40 ^c
	Sesquiterpene hydrocarbons		0.9±0.10 ^d	2.6±0.01 ^c	5.0±0.10 ^a	0.3±0.01 ^e	3.5±0.03 ^b
	Oxygenated sesquiterpenes		-	-	2.7±0.10 ^a	2.0±0.02 ^b	1.0±0.01 ^c
	Essential oil yield % (w/w)		1.85±0.02 ^b	2.3±0.02 ^a	0.75±0.01 ^c	2.25±0.02 ^a	2.50±0.02 ^a
	Total		90.4	90.1	92.45	96.2	96.9

RI: Retention Index; w/w: weight/ weight. Data are expressed as the mean $\pm$ SD (n = 3). ^{a-d} Different lower case letters in superscript within a row indicate significant differences (p < 0.05) between EOs of plants for the same compounds. There is no statistical difference between data that are not lettered in the same row.

2.5 Minimum inhibitory concentration (MIC) of EOs against target bacteria

The evaluation of Minimum Inhibitory Concentration (MIC) values for aerobic bacteria was conducted using the liquid microdilution method, complying with CLSI M07-A10 [13]. Briefly, the essential oils were dissolved in Dimethyl sulfoxide (DMSO) (final concentration of 0.5% (v/v) in Nutrient Broth

(NB)). No detrimental effect on bacterial growth was observed at this concentration. To prepare stock solutions, the obtained essential oils were subjected to a two-fold dilution process. From each essential oil, 50 μ L was added to 130 μ L of NB in each of the 96 wells. Following the addition of 20 μ L of a fresh bacterial suspension (approximately 10⁵ CFU/mL), incubation of the plates was carried out at 37 °C for 24 hours. The concentration range evaluated was 1 - 128 μ L/mL. Each plate

included a sterility control well and a growth control well. Prior to reading, the microplates were shaken at 250 rpm for 10 minutes and absorbance measurements were conducted at 600 nm by using a microplate reader (Epoch, Bio-Tek Instruments, Winooski, Vermont, USA). Minimum Inhibitory Concentration (MIC) was defined as the essential oil concentration that suppressed bacterial growth by 90% compared to the positive control [14].

2.6 Statistical analysis

Each test was conducted three times, and the results were evaluated using one-way ANOVA followed by Tukey's HSD post-hoc test at a 5% significance level via SPSS version 22 (IBM, New York, USA).

3 Results and discussions

3.1 Essential oil compositions

The chemical profile (%) of the EOs of rosemary, sage, basil and two types of mints are presented in Table 1. Oxygenated monoterpenes constituted the predominant group of compounds detected across all species, with *Mentha piperita* L. (90.7%) and *Mentha spicata* L. (78.1%) exhibiting the highest levels. In contrast, monoterpene hydrocarbons were found in varying quantities, being most prominent in *Salvia officinalis* L. (34.1%). Among the identified individual compounds, 1,8-cineole emerged as a predominant constituent across all plant species, with notably high concentrations in *Rosmarinus officinalis* L. (29.7%) and *Salvia officinalis* L. (23.6%). This monoterpene, commonly found in eucalyptus and rosemary oils, exhibits antimicrobial, anti-inflammatory, and antioxidant activities, mainly by modulating NF- κ B and Nrf2 pathways and affecting microbial membrane integrity. These properties make it valuable not only for medicinal applications, such as in respiratory, cardiovascular, and digestive disorders, but also for use in flavoring, fragrance, and cosmetic products. Despite its promising profile, the compound's poor stability, high volatility, poor water solubility and limited pharmacokinetic studies suggest that further research and development of suitable formulations are needed for enhanced therapeutic application [15]. Noteworthy differences were observed for unique compounds such as linalool, dominating in *Ocimum basilicum* L. (53.2%), and menthol, present only in *Mentha piperita* L. (50%). Linalool, a monoterpene alcohol commonly found in essential oils, exhibits a wide spectrum of bioactive properties, including antimicrobial, anti-inflammatory, antihyperlipidemic, antidepressant, neuroprotective, and anticancer effects. Its pharmacological activities are largely mediated through interactions with neurotransmitter receptors and modulation of key signaling pathways such as NF- κ B and Nrf2, leading to reduced production of inflammatory cytokines and reactive oxygen species. Linalool's poor water solubility and high volatility, similar to 1,8-cineole, limit its *in vivo* application, prompting the development of advanced delivery systems, particularly at the nanoscale, to enhance bioavailability and target specificity [16]. Menthol, a cyclic monoterpene alcohol predominantly found in the essential oils of *Mentha canadensis* L. (cornmint) and *M. piperita* L. (peppermint), is responsible for the characteristic cooling and minty aroma of mint plants. Historically used in traditional medicine for treating infections and as an insect repellent, menthol exhibits diverse bioactive properties, including antibacterial, antifungal, analgesic, anaesthetic, chemopreventive, and immunomodulatory effects. Menthol

interacts with microbial and mammalian cell membranes, potentially disturbing their integrity and enhancing membrane permeability, which contributes to both its antimicrobial and penetration-enhancing activities. Its low toxicity, combined with these pharmacological effects, underpins its widespread application in pharmaceutical, cosmetic, food, and tobacco industries. Another prominent unique compound is carvone ($C_{10}H_{14}O$), a ketone monoterpene predominantly found in essential oils of *Mentha* species, which was present at high levels in *Mentha spicata* L. (65%), in close agreement with previous reports indicating concentrations around 67% [17]. This monoterpene exhibits a wide range of bioactive properties, including antimicrobial, antifungal, anticancer, anti-inflammatory, neuroprotective, and antidiabetic effects. Its antibacterial and antifungal activities are largely attributed to its ability to penetrate microbial cells, disrupt membrane integrity, and inhibit biofilm formation. Additionally, carvone has demonstrated antiproliferative effects against multiple cancer cell lines by inducing apoptosis and cell cycle arrest, as well as neuroprotective and metabolic benefits. Beyond medicinal applications, carvone has been incorporated into food packaging, antimicrobial coatings, and nanoparticle formulations to enhance its stability and extend its industrial use.

Twenty-one compounds were found in rosemary, comprising 90.4% of the total essential oil content. 1,8-Cineole (29.7%) and camphor (19.8%) were detected as the major constituents, exhibiting significant antimicrobial and anti-inflammatory properties (Figure 1).

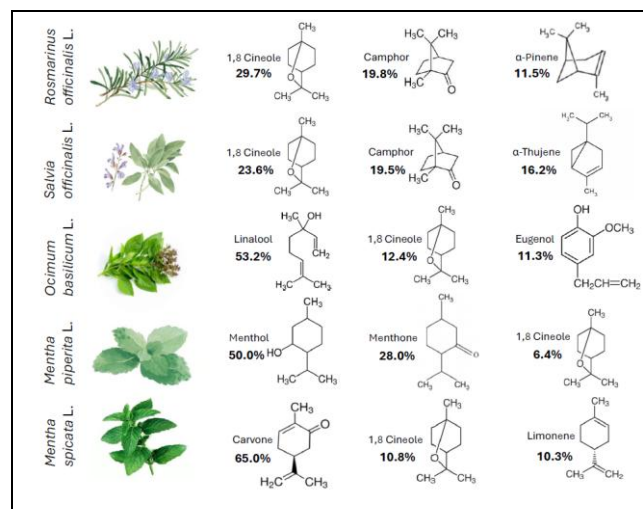


Figure 1. Chemical structures and quantity (%) of the major three compounds in the plants.

Additional components such as borneol (9.3%) and α -pinene (11.5%) further contribute to its applications in aromatherapy and medicinal formulations. In a study investigating essential oils from Tunisian aromatic plants was conducted by Mhiri et al. [18]. *Rosmarinus officinalis* oil, notable differences in the chemical composition are observed. While verbenone (20.99%) was the most abundant compound in Tunisian rosemary, it is absent or present in minimal amounts in our sample. Similarly, camphor, which is a prominent component in both oils, is slightly more abundant in Tunisian rosemary (19.72%) compared to our results (19.8%). These findings emphasize the importance of local adaptation in influencing the phytochemical makeup of medicinal plants.

GC-MS results indicate that oxygenated monoterpenes accounted for 53.4% as the primary class, while monoterpene hydrocarbons contributed 34.1% to the overall composition. In *Salvia officinalis* L., the predominant compounds included 1,8-cineole (23.6%) and camphor (19.5%). These findings align partially with another study, which reported camphor (33.61%) and 1,8-cineole (22.22%) as the major constituents [19]. Also, it is unique among the plants studied for its notable α -Thujene content (16.2%), which is absent in the essential oils of the other species. α -Thujene is a monoterpene hydrocarbon known for its strong antimicrobial and antifungal properties, which likely contribute to the plant's traditional use in preserving food and combating infections [20]. The high concentration of this compound in *Salvia officinalis* L. suggests its potential as a natural preservative or an active ingredient in antimicrobial formulations.

The chemical profile of *Ocimum basilicum* L. was characterized by 22 identified compounds, with linalool (53.2%) being the principal constituent, recognized for its potent antimicrobial and anti-inflammatory properties, followed by eugenol (11.3%), known for its analgesic effects and radical scavenging activity. da Silva et al. [21] identified 65 compounds in basil from northwestern part of the Republic of Srpska, Bosnia and Herzegovina. However, in a different study, methyl chavicol (86.40%) and trans-anethole (8.31%) were the dominant compounds in basil essential oil from northwest Morocco, highlighting the impact of geographical factors on composition [22]. Additionally, 1,8-cineole (12.4%) contributes to the oil's expectorant and respiratory-supportive properties, enhancing its potential applications in treating respiratory conditions. *Mentha piperita* L. and *Mentha spicata* L. exhibited distinct essential oil compositions, highlighting their unique chemical profiles and potential applications. The dominant compound in *Mentha piperita* L. was menthol (50%), followed by menthone (28%) and 1,8-cineole (6.4%), emphasizing its well-known cooling, analgesic, and decongestant properties. These results agree with the results obtained in the studies of Saharkhiz et al. [23] and Beigi, Torki-Harchegani, and Pirbalouti [24]. This profile makes *Mentha piperita* L. particularly suitable for use in respiratory treatments, oral care products, and soothing formulations. In contrast, *Mentha spicata* L. essential oil was notably dominated by carvone, which accounted for 65% of its total constituents and limonene (10.3%), compounds associated with digestive health and antioxidant benefits. Similarly, Chauhan et al. [25] analyzed essential oils from *Mentha spicata* L. in the North-West Himalayan region of India, reporting carvone (76.65%) and limonene (9.57%) as the primary components. Also, in *Mentha spicata* essential oil from the Algerian Saharan Atlas, carvone and limonene were found to be the principal compounds, further confirming their consistent presence across various regions [26]. Unlike *Mentha*

piperita L., *Mentha spicata* L. lacked significant amounts of menthol and menthone, which differentiates its sensory properties and potential uses, favoring applications in flavoring agents and digestive aids. Both species contained β -caryophyllene, though in varying amounts (*Mentha piperita* L.: 0.3%; *Mentha spicata* L.: 2.8%), contributing to their mild anti-inflammatory activities. While *Mentha piperita* L. excels in therapeutic and medicinal use due to its menthol-rich composition, *Mentha spicata* L. is better suited for culinary and digestive applications because of its carvone dominance. These differences underline the versatility of both species in addressing specific industrial and health-related needs.

EO yield data could be explained by differences in climate (rainfall, temperature), soil properties (pH, organic matter), and possibly altitude, depending on where the plant samples were collected. In the current study, the lowest EO yield was obtained from *Ocimum basilicum* (0.75%), whereas *Mentha spicata* exhibited the highest yield (2.50%) among all analyzed species. In a study, *Rosmarinus officinalis* harvested monthly over a two-year period (2018-2019) from three different locations in the Mediterranean region showed significant variations in essential oil yield depending on both the collection site and the harvest time [27]. Similarly, seasonal variation markedly influenced *Salvia officinalis*, with essential oil yield being higher during the summer months [28]. Furthermore, soil amendments (e.g., use of organic manure) in plants have been shown to influence oil quality and yield [29],[30]. In conclusion, the essential oil composition is shaped by genetic traits, environmental factors like climate and light, and agronomic practices such as harvest timing and plant density.

3.2 Antimicrobial activity

The antimicrobial properties of the essential oils were evaluated using the agar-well diffusion assay and broth dilution method (Table 2). Among all tested bacteria, the lowest MIC value was observed for the essential oil derived from *Mentha piperita* L., which aligns with the findings of Mhiri et al. [18], who observed that *Mentha piperita* possesses notable antibacterial properties effective against both Gram-positive and Gram-negative bacteria, potentially attributed to the presence of bioactive compounds. As indicated before, the major compound of *Mentha piperita* was menthol (50.0%), followed by menthone (28.0%) as the second most abundant constituent. In a study investigating the antimicrobial activity of twenty-one oxygenated monoterpenes against 63 different bacterial strains, pure menthol was reported to exhibit inhibition in 24 strains, indicating its strong antimicrobial potential [31].

Table 2. Inhibitory properties of essential oils against the bacterial isolates.

Strains	MIC (μ L/mL)					Zone Diameter (mm) (15 μ L)					G	K
	R.o	S.o	O.b	M.p	M.s	R.o	S.o	O.b	M.p	M.s		
<i>E. c</i>	32	<1	4	<1	<1	11.55 $\pm$ 1.24 ^d	11.53 $\pm$ 0.40 ^{dB}	11.48 $\pm$ 1.30 ^{dB}	16.18 $\pm$ 0.60 ^{CB}	12.09 $\pm$ 0.91 ^{DA}	26.50 $\pm$ 1.35 ^a	21.47 $\pm$ 0.18 ^{BB}
<i>S. a</i>	64	16	16	4	8	11.65 $\pm$ 0.22 ^c	13.10 $\pm$ 0.01 ^{CA}	13.47 $\pm$ 0.93 ^{CA}	19.25 $\pm$ 0.92 ^{BA}	12.72 $\pm$ 1.20 ^{CA}	26.29 $\pm$ 1.87 ^a	21.15 $\pm$ 0.84 ^{BB}
<i>L. m</i>	32	64	32	16	128	ND	ND	10.55 $\pm$ 0.43 ^{BB}	10.92 $\pm$ 0.77 ^{BC}	8.76 $\pm$ 1.03 ^{BB}	23.98 $\pm$ 1.41 ^a	23.72 $\pm$ 0.92 ^{AA}

E.c: *E. coli* O157:H7 NTCC12900; S.a: *S. aureus* ATCC 29213; L. m: *L. monocytogenes* ATCC 7644; R.o: *Rosmarinus officinalis* L.; S.o: *Salvia officinalis* L.; O.b: *Ocimum basilicum* L.; M.p: *Mentha piperita* L.; M.s: *Mentha spicata* L.; G: Gentamycin; K: Kanamycin. ^{a-d} Different lower case letters in superscript within a row indicate significant differences ($p < 0.05$) between EOs of plants for the same bacteria. ^{A-C} Different upper case letters in superscript within a column indicate significant differences ($p < 0.05$) between bacteria for the same EOs of plants. There is no statistical difference between data that are not lettered.

The MIC value for *Mentha piperita* EO against *L. monocytogenes* ATCC 7644 was found to be 16 µg/mL, significantly lower than that of *Mentha spicata* L., which exhibited a MIC of 128 µg/mL. Such findings highlight the potential of *Mentha piperita* L. as a more effective antimicrobial agent against *L. monocytogenes* ATCC 7644 compared to *Mentha spicata* L., suggesting variations in the chemical composition of the essential oils and their corresponding antibacterial properties. The weak and limited antibacterial activity of *Mentha spicata* can be attributed to its high carvone content (65%), which is consistent with the lower antimicrobial efficacy observed compared to *Mentha piperita* [31]. Kang et al. [14] reported that the MIC value of peppermint EO against *S. aureus* 0.5 mg/mL. In contrast, our findings indicate a considerably higher MIC value of 4 µL/mL. This discrepancy could be attributed to differences in experimental conditions, such as the composition of the essential oil, variations in bacterial strains, or methodological approaches.

In the present study, the highest MIC value was recorded for *Rosmarinus officinalis* L. against *S. aureus* ATCC 29213 (64 µL/mL), the result is similar to those reported by Endo et al. [32], with MIC value of 62.5 µg/mL against same strain of *S. aureus*. *L. monocytogenes* ATCC 7644 exhibited the highest resistance among those tested, similar to the approach taken by Mazzarrino et al. [33]. They found the MIC value higher than 40 µg/mL for *Salvia officinalis* EOs, whereas our study determined the MIC value to be 64 µg/mL. Consistent results were observed in the agar-well diffusion analysis. *E. coli* O157:H7 NTCC12900 was identified as the most sensitive bacteria to essential oils of *Salvia officinalis* L., *Mentha piperita* L. and *Mentha spicata* L. used in this study. Conversely, literature indicates that Gram-positive bacteria typically exhibit higher susceptibility to hydrophobic antimicrobial agents such as essential oils (EOs) compared to Gram-negative bacteria, largely due to differences in their cell-wall architecture. Gram-positive bacteria possess a thick, exposed peptidoglycan layer, often interlinked with teichoic and lipoteichoic acids, which may facilitate easier penetration of hydrophobic EO constituents. In contrast,

Gram-negative bacteria are characterised by a more complex envelope: an outer membrane comprised of lipopolysaccharides (LPS), phospholipids and proteins overlays a much thinner peptidoglycan layer, creating an additional barrier that restricts diffusion of lipophilic compounds [34]

According to the classification indicated by Denev et al. [35], the results of the agar well diffusion assay were categorized into three groups based on inhibition zone diameter: weak (<12 mm), moderate (12-20 mm), and strong (>20 mm). Both gentamicin and kanamycin exhibited strong inhibitory effects against all tested microorganisms. Apart from *L. monocytogenes* ATCC 7644, the largest inhibition zones for the other two bacteria were observed first with gentamicin, followed by kanamycin ($p < 0.05$). Notably, *Mentha piperita* L. demonstrated an antimicrobial effect against *S. aureus* ATCC 29213 that was comparable to kanamycin. Overall, the finding that EOs of *Mentha piperita* L. was the most effective plant EOs after gentamicin and kanamycin is highly promising and highlights its potential as a natural antimicrobial agent (Figure 2). This activity could be attributed to menthol, the active compound in peppermint. Against *S. aureus* ATCC 29213 and *E. coli* O157:H7 NTCC12900 moderate activities were observed for EOs of *Mentha piperita* L. Similarly, Tyagi and Malik [36] observed a zone of inhibition of 16 mm for *E. coli* ATCC 25922, demonstrating the consistent antibacterial potential of *Mentha piperita* essential oil against different *E. coli* strains. *L. monocytogenes* ATCC 7644 strain was identified as the most resistant bacteria to all essential oils. No zone formation was detected against the essential oils of *Rosmarinus officinalis* and *Salvia officinalis* L. Unlike the findings of Jordán et al. [37], who reported strong inhibition zones (≥ 20 mm) for most chemotypes of *Rosmarinus officinalis* against *L. monocytogenes* ATCC 7644, our results indicate that these essential oils showed no detectable antibacterial effects against this strain, highlighting potential differences in susceptibility depending on strain type or experimental conditions.

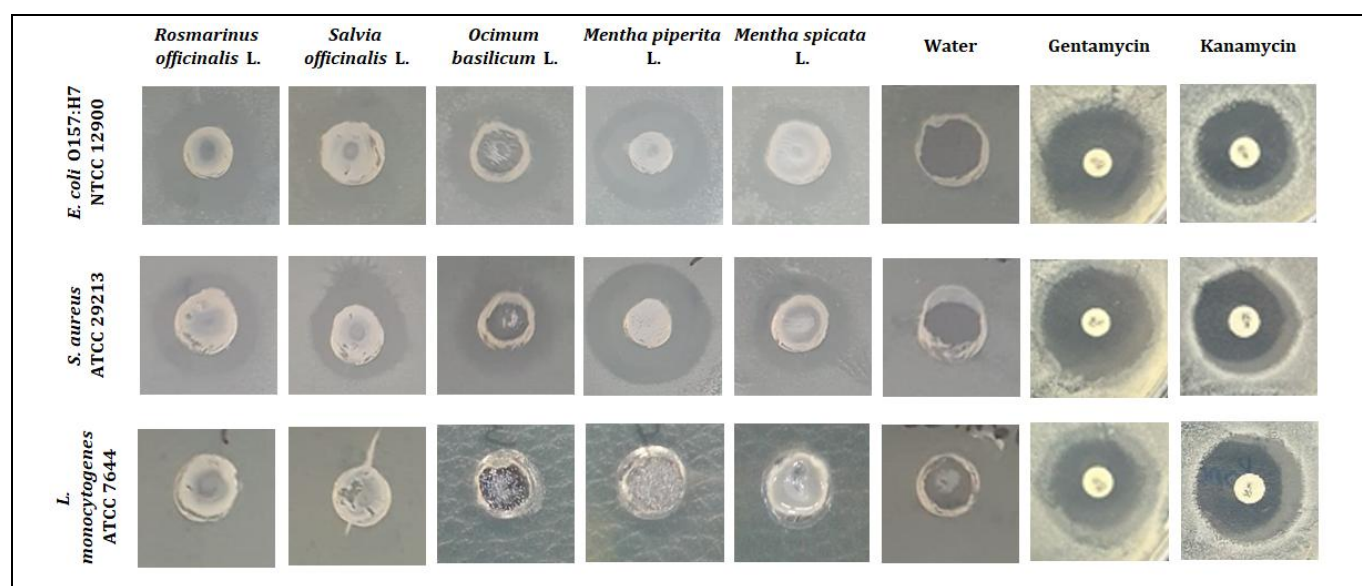


Figure 2. The inhibition zone diameter of tested bacteria against essential oils and antibiotics.

Also, in a study where the inhibition zone diameters of twenty-one pure oxygenated monoterpenes were evaluated against 63 different bacterial strains, 1,8-cineole and camphor were reported to produce no inhibition zones in any of the tested bacteria [31]. This observation can explain why *Rosmarinus officinalis* and *Salvia officinalis*, which contain more than 40% of these compounds, did not exhibit any inhibition against *L. monocytogenes* in our study.

4 Conclusion

The chemical constituents and antimicrobial efficacy of essential oils (EOs) from rosemary, sage, basil, peppermint, and spearmint were comprehensively analyzed in this research. The findings demonstrate that oxygenated monoterpenes are the principal chemical components identified across all species, with notable variations in individual compound profiles. For instance, *Mentha piperita* L. exhibited a menthol-rich profile (50%), highlighting its suitability for therapeutic applications, while *Mentha spicata* L., characterized by high carvone content (65%), is better suited for digestive and culinary uses. Similarly, basil essential oil was dominated by linalool (53.2%), known for its antimicrobial properties, whereas rosemary and sage essential oils were rich in 1,8-cineole and camphor, compounds with recognized medicinal and aromatic applications. The antimicrobial assays revealed significant variability in activity among the EOs tested. *Mentha piperita* L. displayed the strongest antimicrobial potential, attributed to its high menthol content. Conversely, *Rosmarinus officinalis* L. and *Salvia officinalis* L. exhibited weaker or no detectable activity against certain strains, emphasizing the influence of chemical composition on antimicrobial efficacy. These findings align with previous research while also highlighting geographical differences and variations arising from differences in extraction and analytical methodologies affecting essential oil composition and bioactivity.

The advancement of essential oil formulation and targeted delivery methodologies to improve bioactivity, alongside the management of sensory effects, should constitute a key aspect of future research. Continued exploration of essential oils in innovative applications holds significant promise for improving food safety and contributing to the development of eco-friendly antimicrobial solutions.

5 Acknowledgment

The authors wish to thank Bursa Technical University, Bursa Uludag University and Central Research Institute of Food and Feed Control for supporting laboratory facilities.

6 Author contribution statements

Seda Altuntas, conceptualization, methodology, formal analysis, investigation, data curation, visualization, writing original draft, writing-review and editing. Ayca Yigit Cinar, conceptualization, methodology, investigation, writing-review and editing and project administration. Oya Kacar, methodology, formal analysis, investigation, data curation, writing-review and editing. Sema Demir: methodology, formal analysis, investigation, data curation, writing-review and editing. All authors have read and agreed to the published version of the manuscript.

7 Ethics committee approval and conflict of interest statement

"Ethics committee approval is not required for the prepared manuscript". "There is no conflict of interest with any individual or institution in the prepared manuscript".

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