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Antioxidant activity, chemical composition, and therapeutic activity of essential oils from certain Lamiaceae species in Türkiye

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ABSTRACT: The study examined the antioxidant, antimicrobial, and potential anticancer properties of lavandin (Lavandula x intermedia 'Grosso'), oregano (Origanum vulgare 'Uluğ Bey'), and sage (Salvia officinalis 'Beyhekim') essential oils (EOs). Chemical analysis revealed that oregano is rich in carvacrol (81.23%), cymene (5.51%), and 18-cineole (2.13%). Sage contains notable quantities of α-thujone (17.47%), camphor (13.59%), and borneol (12.14%). Lavandin is predominantly composed of linalool (41.10%), linalyl acetate (16.66%), and camphor (10.01%). The antimicrobial tests demonstrated that lavandin EOs exhibited a high inhibition rate of 90.77% at 1000 µg/mL, which subsequently decreased with lower concentrations. The oregano essential oils demonstrated robust antimicrobial activity across all concentrations, with a 10.97% inhibition rate observed even at the lowest concentration. The sage EOs exhibited a rapid decline in antimicrobial activity, reaching 4.97% at the lowest concentration. Both the DPPH and ABTS assays demonstrated that the antioxidant capacity of the essential oils was concentration-dependent. The cytotoxicity values of oregano essential oil were 35.58 at 50 µg/mL, 32.39 at 25 µg/mL, and 30.92 at 12.5 µg/mL. Similar cytotoxicity results were observed for sage and lavandin essential oils. These findings indicate that plant essential oils exert a dose-dependent cytotoxic effect on cell growth, significantly inhibiting cells at high concentrations. The research indicates that essential oils derived from Lamiaceae plants exhibit robust antimicrobial effects against a range of pathogens, suggesting potential applications as alternative therapies for specific microbial infections, comparable to ciprofloxacin at certain concentrations.

KEYWORDS: *Origanum vulgare* subs. *hirtum*; *Salvia officinalis* L., *lavandula* x *intermedia*; antioxidant; antimicrobial, cytotoxicity.

1. INTRODUCTION

The Lamiaceae family, popularly known as the mint family, consists of around 250 genera and 7825 species worldwide. The Lamiaceae family comprises 46 genera and about 725 species in Türkiye [1-4]. The Lamiaceae family contains various species that are highly appreciated for their use in food, medicine, and cosmetics [5]. The leaves and flowers of the Lamiaceae family contain quality Essential oils (EOs). These EOs have antibacterial, antifungal, antioxidant, antiviral, and anticancer properties, making them useful in medicine, perfumery, and cosmetics [6-8].

Sage (*Salvia officinalis* L.) is an evergreen plant with numerous medicinal benefits. It is the largest genus of plants in the Lamiaceae family, with about 900 species [9-10]. Sage is found in three distinct regions: the Mediterranean, Iran, and some sections of Europe. This plant has long been used in traditional medicine, primarily to improve cognition and treat colds, bronchitis, cardiovascular diseases, gastrointestinal disorders, cancers, excessive sweating, depression, nervous disorders, and cerebral ischemia [9,11-13]

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tuberculosis [14]. It has been shown to have a variety of therapeutic properties, including antioxidant, antibacterial, anti-inflammatory, hypoglycemic anti-dementia, anticancer, and hypolipidemic actions [15-18]. Sage's antioxidant and antibacterial effects have been related to the presence of camphor, thujone, and 1,8-cineole [19].

Sage is also regarded as an aromatic plant, which means it contains a high concentration of EO (0.7-5.2%). EOs, which are mostly composed of volatile organic molecules known as terpenes, are also well-known and extensively researched due to their biological activity, which includes antioxidant, antibacterial, antivirulent, antiparasitical, and insecticidal properties [10]. Sage EOs contain approximately 120 compounds, with the main ones being eucalyptol (1,8-cineole], thujenes (α- and β-thujenes), and borneol, as well as sesquiterpenes α-caryophyllene (humulene) and β-caryophyllene [20].

The *Origanum* genus contains various species that differ in shape and chemical properties, with the majority of them originating in the Region of the Mediterranean [21]. The *Origanum* genus has gained popularity as a spice plant due to its antifungal, antimicrobial, and antioxidant qualities [22]. One of the most noteworthy species is Istanbul Oregano, *Origanum vulgare* subsp. *hirtum*, a commonly used aromatic plant in foods, feeds, cosmetics, and personal care items, owing to its EOs, which are primarily high in carvacrol and thymol [23]. Compared to other *Origanum* species, *Origanum vulgare* subsp. *hirtum*, which is frequently found in Turkish flora, has been distinguished by its high EO content. The *Origanum* genus, which includes 21 species, 24 taxa, and 13 hybrids, is abundant in the Turkish flora. This genus is 67% endemism in Türkiye. [24,25]. Among aromatic herbs, Istanbul oregano EOs have some of the strongest antioxidant effects [22].

The Lamiaceae plant family includes the genus *Lavandula*, commonly known as lavender, which has about 39 recognized species, subspecies, and hybrids [26]. Several regions of the world, including the Mediterranean, northern Africa, southwest Asia, Arabia, and western Iran, are home to endemic species of *Lavandula* (L.). Through traditional breeding techniques, new cultivars have been created to enhance the concentration of particular chemical compounds in plants. For example, fertile *Lavandula x intermedia* cultivars have been produced by crossbreeding *Lavandula angustifolia* (lavender) and *Lavandula latifolia* [27,28]. The cultivar *Lavandula x intermedia* (lavandin) 'Grosso' is the most well-known for its EOs product and odor among the three main lavandin types (Abrial, Super, and Grosso) Furthermore, some other characteristics of lavandin Grosso EOs include phytotoxicity, antiplatelet/antithrombotic, antibacterial, antioxidant, and antifungal activities [29-31].

The objective of this study is to examine the antioxidant, antibacterial, and cytotoxic activities of essential oils derived from *Salvia officinalis, Origanum vulgare subsp. hirtum*, and *Lavandula x intermedia* (Lavandin) plants. The research seeks to elucidate the potential therapeutic applications of these essential oils and their positive effects on health. Additionally, the study assesses the bioactive properties of these essential oils to evaluate their potential in managing and treating various health issues.

2. RESULTS

2.1. Characterization

A high concentration of carvacrol (81.23%) was found in the chemical composition of *Origanum vulgare* subsp. *hirtum*. Carvacrol is a significant component responsible for the plant's antioxidant and antimicrobial properties. The high percentage of carvacrol suggests that the plant may have potential health benefits. Additionally, *Origanum vulgare* subsp. *hirtum* contains other constituents such as cymene (5.51%) and gamma-terpinene (2.13%) (Table 1). It is recognized that these components may have positive effects on plant health. For instance, 1,8-cineole is known to have respiratory soothing and anti-inflammatory properties. The chemical composition of *Origanum vulgare* subsp. *hirtum* can help us understand the potential health benefits of the plant. The high percentages of constituents with antioxidant, antimicrobial, and anti-inflammatory properties suggest that the plant may have health-promoting properties [32,33].

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Table 1. Chemical composition of *Origanum vulgare* subsp. *hirtum*

Peak	Retention time	Retention indice	Compound	%
1	12.898	1021	α-pinene	0.86
2	13.046	1024	α-thujene	1.26
3	14.793	1108	camphene	0.55
4	16.636	1160	β-pinene	0.18
5	19.055	1065	β-myrcene	1.21
6	19.891	1179	α-terpinene	0.71
7	20.748	1197	limonene	0.21
8	21.207	1208	β-phellandrene	0.21
9	22.792	1245	gamma-terpinene	2.13
10	23.896	1270	cymene	5.51
11	30.341	1437	1-octen-3-ol	0.33
12	31.051	1457	cis-sabinene hydrate	0.50
13	33.875	1542	trans-sabinene hydrate	0.28
14	35.729	1599	carvacrol methyl ether	1.19
15	35.873	1604	β-caryophyllene	1.18
16	38.683	1699	borneol	1.35
17	39.419	1725	β-bisabolene	0.47
18	39.915	1742	carvone	0.16
19	46.8	2003	caryophyllene oxide	0.21
20	50.832	2170	thymol	0.30
21	51.653	2204	carvacrol	81.23

The chemical composition of *Salvia officinalis* contains noteworthy percentages of certain constituents. For instance, *Salvia officinalis* contains high percentages of *α*-thujone (17.472%), camphor (13.594%), and borneol (12.143%). These constituents are believed to have significant effects on the plant's potential health benefits. Other constituents found in *Salvia officinalis* include linalool (0.449%), *β*-caryophyllene (0.629%), and viridiflorol (4.853%) (Table 2). These components are also believed to have beneficial properties for health. For instance, linalool is known for its calming effects and may help reduce stress [34].

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Table 2. Chemical composition of *Salvia officinalis*

Peak	Retention time	Retention indice	Compound	%
1	10.15	*955*	*Cis*-salvene	0.484
2	13.169	*1027*	*α*-pinene	6.52
3	15.103	1072	camphene	6.403
4	16.969	*1115*	*β*-pinene	6.581
5	19.377	*1167*	*β*-myrcene	0.764
6	21.087	1205	limonene	1.497
7	21.469	1214	1,8-cineole	14.38
8	22.536	*1239*	*Cis*-ocimene	0.544
9	23.131	*1252*	*γ*-terpinene	0.381
10	24.227	1278	cymene	0.265
11	24.723	1290	terpinolene	0.23
12	30.219	*1434*	*α*-thujone	17.472
13	30.884	*1453*	*β*-thujone	4.215
14	33.619	1534	camphor	13.594
15	33.852	1541	linalool	0.449
16	35.439	1590	bornyl acetate	0.768
17	35.976	1608	terpinene-4-ol	0.435
18	36.211	*1616*	*β*-caryophyllene	0.629
19	37.993	*1675*	*δ*-terpineol	0.27
20	38.429	*1690*	*α*-humulene	3.373
21	38.709	*1699*	*α*-terpineol	0.294
22	38.778	1702	3-thujanol	0.289
23	38.845	*1704*	*γ*-muurolene	0.315
24	38.97	1709	borneol	12.143
25	40.763	1772	δ-cadinene	0.366
26	48.446	2071	humulene epoxide II	0.509
27	49.231	2103	viridiflorol	4.853
28	69.326	2615	manool	1.978

Table 3. Chemical composition of *Lavandula x intermedia*

Peak	Retention time	Retention indice	Compound	%
1	11.49	1027	α-pinene	0.46
2	13.21	1072	camphene	0.38
3	14.95	1115	β-pinene	0.53
4	17.34	1169	myrcene	0.81
5	18.99	1206	limonene	0.65
6	19.51	1218	1,8-cineole	8.90
7	20.47	1241	β-ocimene	1.10
8	22.57	1290	α-terpinolene	0.30
9	28.8	1454	1-octen-3-ol	0.48
10	31.64	1539	camphor	10.01
11	32.11	1554	linalool	41.10
12	32.53	1567	linalyl acetate	16.66
13	34.07	1616	lavandulyl acetate	2.83
14	34.12	1618	terpinen-4-ol	3.17
15	35.82	1675	trans-β-farnesene	0.37
16	36.1	1684	lavandulol	0.65
17	36.89	1711	α-terpineol	3.39
18	37.04	1716	borneol	2.97
19	37.61	1737	neryl acetate	0.51
20	38.46	1767	geranyl acetate	1.21
21	39.61	1808	nerol	0.60
22	40.81	1853	geraniol	1.47
23	49.21	2144	epi bicyclosesquiphellandrene	0.54
24	50.11	2205	α-bisabolol	0.92

The lavandin plant's chemical composition reveals notable percentages of certain components. The lavandin plant contains various components, with the highest percentages being linalool (41.10%), linalyl acetate (16.66%), and camphor (10.01%). It is thought that these components may have significant effects on the plant's potential health benefits. Other components found in the plant include α-terpineol (3.39%), borneol (2.97%), and geraniol (1.47%) (Table 3). These components are believed to have beneficial properties for the plant's health. For instance, α-terpineol is recognized for its antioxidant and antimicrobial properties. The lavandin plant's chemical composition offers valuable insights into its potential health benefits. High percentages of ingredients with antioxidant, anti-inflammatory, and calming properties suggest that Lavandin may have health-supporting properties [35,36].

2.2. Radical Scavenging Activity

2.2.1 DPPH• Radical Scavenging Activity

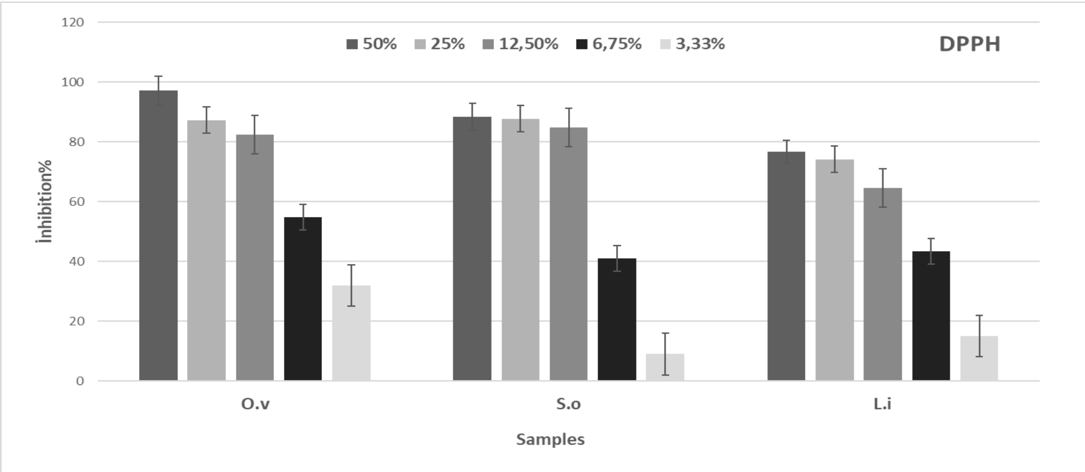

Figure 1. Antioxidant activity (Inhibition %) values of *O. vulgare* subsp. *hirtum*, *S. officinalis* and *Lavandula x intermedia*, n=3, O.v: *Origanum vulgare* subsp. *hirtum*; S.o: *Salvia officinalis*, L.i: *Lavandula x intermedia*; DPPH: 1,1-difenil2-pikrilhidrazil

Figure 1 presents quantitative values for the antioxidant capacities of oregano, sage, and lavandin EOs at varying concentrations. These values are essential in comprehending the radical scavenging activities of the plant EOs and their potential as antioxidants. For example, at a concentration of 50 µg/mL, oregano EO demonstrated an antioxidant capacity of 97.20%, while sage and lavandin EOs exhibited values of 88.42%, and 76.72%, respectively. As the concentration decreased to 25 µg/mL, the antioxidant capacities slightly decreased for all EOs. The results indicate that there is a concentration-dependent relationship between the concentration of the EOs and their antioxidant capacities. Further decrease in concentration to 12.5 µg/mL resulted in a noticeable decrease in the antioxidant capacities of oregano, sage, and lavandin EOs. This trend suggests that the antioxidant potential of the EOs diminishes as the concentration decreases. These results are crucial for comprehending the radical scavenging abilities of the EOs and their effectiveness in neutralizing free radicals. The concentration-dependent changes in antioxidant capacities emphasize the significance of dosage considerations when using these EOs for their antioxidant properties.

2.2.2 ABTS•+ Radical Scavenging

The ABTS radical scavenging capacity is widely used to determine antioxidant activity. This method measures the ability of antioxidant compounds to neutralize free radicals and determine their antioxidant potential. Figure 2 shows the ABTS radical scavenging activity values for different concentrations of oregano, sage, and lavandin EOs, reflecting the antioxidant capacities of the plants. The results demonstrate that the radical scavenging activity increases as the concentrations of oregano, sage, and lavandin increase. This suggests that the antioxidant activities of the EOs are concentration-dependent. Higher concentrations exhibit a more pronounced radical scavenging activity. Oregano EO showed values of 2.29, and 2.44 mmol/L Trolox, at concentrations of 50 µg/mL and 25 µg/mL, respectively. Similar results were obtained for sage and lavandin EOs at varying concentrations (Figure 2). Upon reviewing the results, it was observed that the ABTS radical scavenging activity generally increased with increasing concentrations. For example, a significant increase in radical scavenging activity was observed with increasing concentration of oregano EO. This suggests that the antioxidant capacities of the plant EOs may vary depending on concentration. The results indicate that the ABTS method is an effective tool for evaluating the antioxidant activities of oregano, sage, and lavandin. The higher radical scavenging activity at higher concentrations suggests that the antioxidant potential of the plant EOs may increase [37]. The results obtained show that DPPH and ABTS methods are compatible with each other.

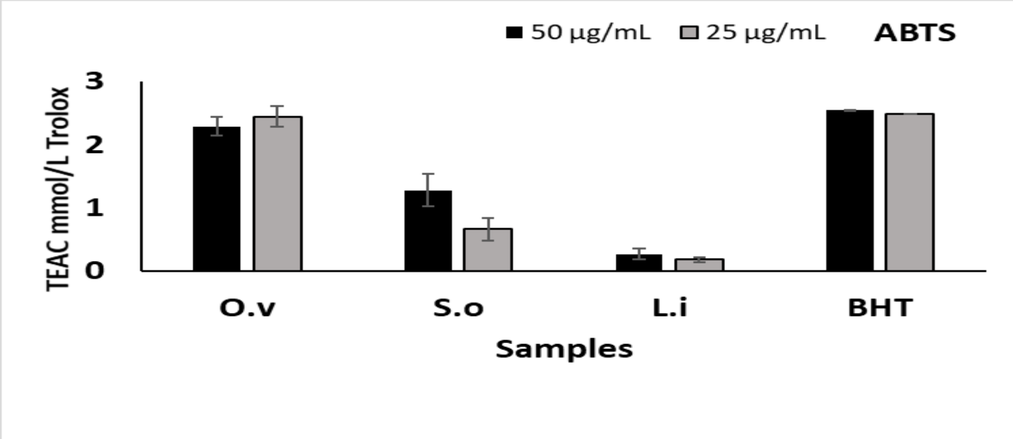

Figure 2. Antioxidant activity (TEAC mmol/L Trolox)) values of *O. vulgare* subsp. *hirtum*, *S. officinalis*, and *Lavandula x intermedia*, n=3, O.v: *Origanum vulgare* subsp. *hirtum*; S.o: *Salvia officinalis*, L.i: *Lavandula x intermedia*; ABTS: 2,2′-Azino-Bis(3-Etilbenztiyazolin-6-Sülfonik Asit); BHT: Butylated hydroxytoluene

2.3. Cytotoxicity

Figure 3 shows the effects of oregano, sage, and lavandin EOs on Hela cell line growth at different concentrations. Higher concentrations resulted in increased cytotoxicity values, such as 35.58 at 50 µg/mL, 32.39 at 25 µg/mL, and 30.92 at 12.5 µg/mL for oregano EOs. Similarly, the cytotoxicity values for sage and lavandin EOs were also observed to be similar at different concentrations. These results suggest that oregano, sage, and lavandin EOs have a dose-dependent cytotoxic effect on cell growth. The increasing cytotoxicity at higher concentrations indicates that the EOs may have a significant inhibitory effect on cells.

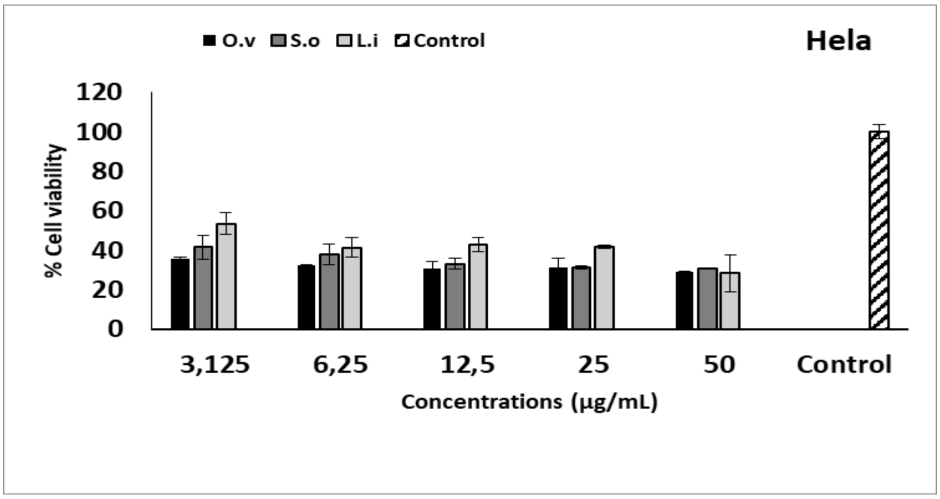

Figure 3. Cell viability (%) values of *O. vulgare* subsp. *hirtum* *S. officinalis* and *Lavandula x intermedia*, n=3, O.v: *Origanum vulgare* subsp. *hirtum*; S.o: *Salvia officinalis*, L.i: *Lavandula x intermedia*

In addition, the fluctuation of cytotoxicity values with concentration indicates that plant EOs may have varying effects on cells, and dosage is a critical factor. Therefore, it is essential to make careful dosage adjustments when using plant EOs, considering their potential toxic effects [38].

2.4. Antibacterial effect

The study evaluated the antibacterial activity of lavandin, oregano, and sage EOs, as well as ciprofloxacin, on various bacterial strains, including *Acinetobacter* sp., *Acinetobacter baumannii* ATCC 17606 standard strain, Methicillin-Resistant *Staphylococcus aureus* sp. (MRSA sp.), MRSA (LY 1999 0053) standard strain, *Klebsiella* sp., and *Klebsiella pneumoniae* ATCC 4352 standard strain, at different concentrations. The results demonstrate the ability of each component to inhibit bacterial growth at specific concentrations.

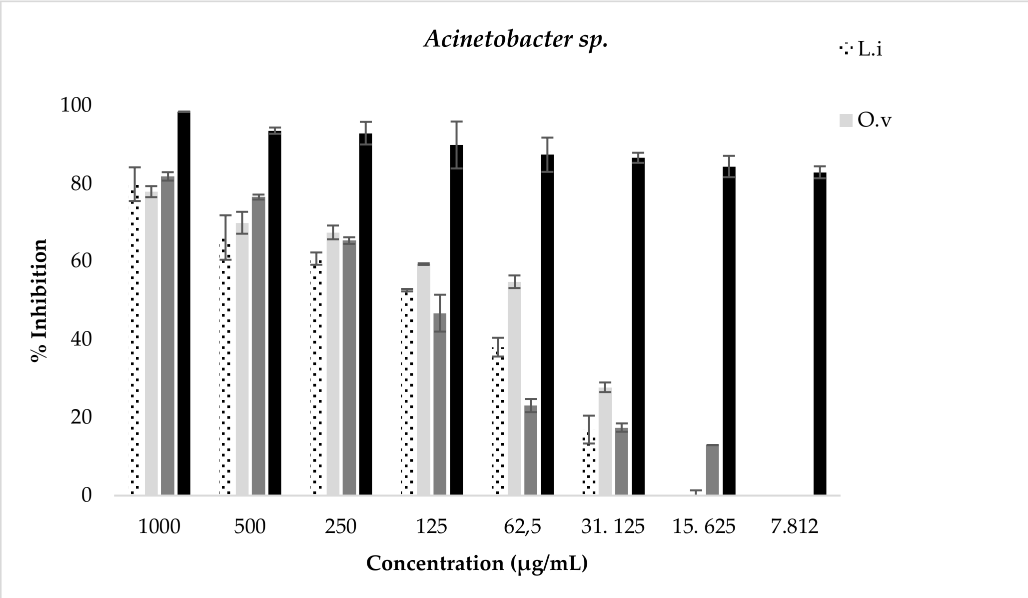

Figure 4. Inhibitory effect of L.i, O.v, and S.o with respective concentrations towards *Acinetobacter sp.,* n=3, O.v: *Origanum vulgare* subsp. *hirtum;* S.o: *Salvia officinalis*, L.i: *Lavandula x intermedia*

At a concentration of 1000 µg/mL, lavandin EOs exhibited a very high inhibition rate of 79.89% against *Acinetobacter* sp., which decreased as the concentration decreased. At 31.25 µg/mL, the inhibition rate was 16.94%. This suggests that the antibacterial activity of lavandin EOs was concentration-sensitive and decreased at lower concentrations. Oregano EOs, on the other hand, demonstrated a strong antibacterial effect low concentration levels, with an impressive initial inhibition rate of 77.98% at 1000 µg/mL. Although the activity decreased as the concentration decreased, it still exhibited an acceptable inhibition rate of 27.79% at 31.125 µg/mL. The ability of oregano EOs to maintain antibacterial activity over a wide concentration range highlights the potential of these EOs. Sage EOs exhibited an inhibition rate of 81.89% at a concentration of 1000 µg/mL, but this effect decreased significantly as the concentration decreased, reaching 412.95% at 15.625 µg/mL (Figure 4). The study found that the antibacterial effect of sage EOs decreases rapidly as the concentration decreases. Ciprofloxacin demonstrated more consistent antibacterial activity than the other substances examined. The antibiotic exhibited an extremely high inhibition rate of 98.48% at 1000 µg/mL and a strong inhibition rate of 82.95% even at the lowest concentration of 7.812 µg/mL. These results demonstrate that ciprofloxacin is highly effective against *Acinetobacter* sp. across a wide range of concentrations.

At a concentration of 1000 µg/mL, lavandin EOs exhibited a very high inhibition rate of 90.77%, which decreased as the concentration decreased. At 31.25 µg/mL, the inhibition rate was 26.95%. This suggests that the antibacterial activity of lavandin EOs is concentration-sensitive and decreases at lower concentrations. Oregano EOs, on the other hand, demonstrated a strong antibacterial effect at all concentration levels, with an impressive initial inhibition rate of 97.45% at 1000 µg/mL. Although the activity decreased as the concentration decreased, it still exhibited an acceptable inhibition rate of 10.97% at 7.812 µg/mL. The ability of oregano EOs to maintain antibacterial activity over a wide concentration range highlights the potential of these essential EOs. Sage EOs exhibited an inhibition rate of 82.45% at a concentration of 1000 µg/mL, but this effect decreased significantly as the concentration decreased, reaching 4.97% at 7.812 µg/mL. The study found that the antibacterial effect of sage EOs decreases rapidly as the concentration decreases. Ciprofloxacin demonstrated more consistent antibacterial activity than the other substances examined. The antibiotic exhibited an extremely high inhibition rate of 99.11% at 1000 µg/mL and a strong inhibition rate of 82.92% even at the lowest concentration of 7.812 µg/mL (Figure 5). These results demonstrate that ciprofloxacin is highly effective against *Acinetobacter baumannii* across a wide range of concentrations.

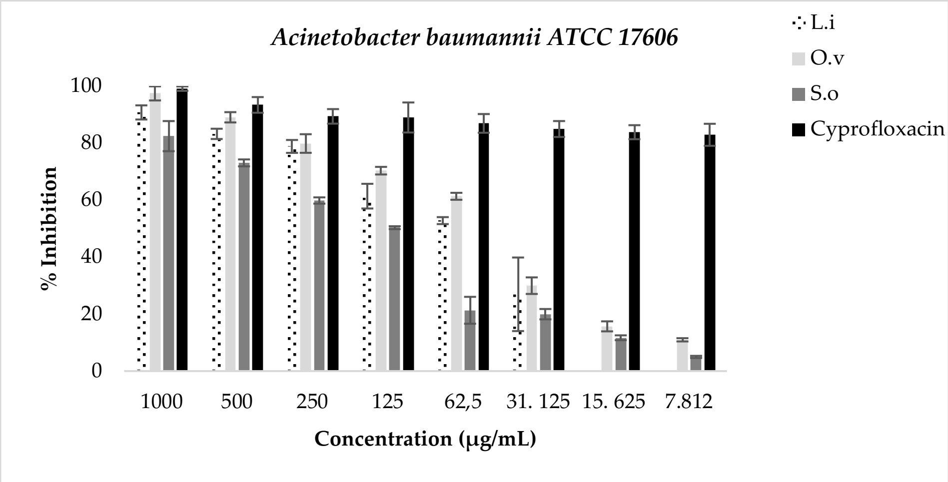

Figure 5. Inhibitory effect of L.i, O.v, and S.o with respective concentrations towards *Acinetobacter baumannii*, n = 3, O.v: *Origanum vulgare* subsp. *hirtum*; S.o: *Salvia officinalis*, L.i: *Lavandula x intermedia*

At a concentration of 1000 µg/mL, lavandin EOs demonstrated an impressive initial inhibition rate of 89.59%. However, as the concentration decreased, the rate also decreased and reached 31.45% at 31.25 µg/mL. Lavandin EOs did not show any inhibitory effect on MRSA sp. at concentrations of 15.625 µg/mL and below. Oregano EOs did not exhibit any inhibition at 15.635 µg/mL but showed an effect of 77.01% at 1000 µg/mL. The antibacterial effect of oregano EOs on MRSA sp. decreased as the concentration decreased, reaching 16.98% at 31.25 µg/mL. It is evident that both EOs are effective against MRSA sp. at moderate concentrations, but their effectiveness decreases at lower concentrations. Similarly, sage EOs exhibited an inhibition of 65.26% at a concentration of 1000 µg/mL, which decreased to 7.34% at 31.25 µg/mL. The study found that sage EOs were effective against MRSA sp. within a specific concentration range, but their effectiveness decreased significantly at lower concentrations. Ciprofloxacin consistently exhibited higher inhibition rates than other substances tested in the study. This antibiotic had an initial inhibition rate of 92.79% at 1000 µg/mL and maintained an impressive inhibition rate of 77.18% even at the lowest concentration of 7.812 µg/mL (Figure 6). These findings demonstrate that ciprofloxacin has strong and consistent antibacterialactivity across a wide range of concentrations, making it an effective agent against MRSA sp.

On the MRSA (LY 1999 0053) standard strain, lavandin EOs were highly effective at the highest concentration, inhibiting growth by 73.89%. However, its effectiveness decreased rapidly at lower concentrations, indicating that lavandin EOs were only effective above a certain concentration level against MRSA LY 1999 0053 standard strain. Oregano EOs also showed a significant effect on the MRSA (LY 1999 0053) standard strain at high concentrations, inhibiting growth by 64.99%. However, its effectiveness decreased rapidly at lower concentrations, indicating that lavandin EOs were only effective against MRSA LY 1999 0053 standard strain above a certain concentration level. Sage EOs demonstrated the highest inhibition rate (91.89%) against this strain and retained its effectiveness even at lower concentrations.

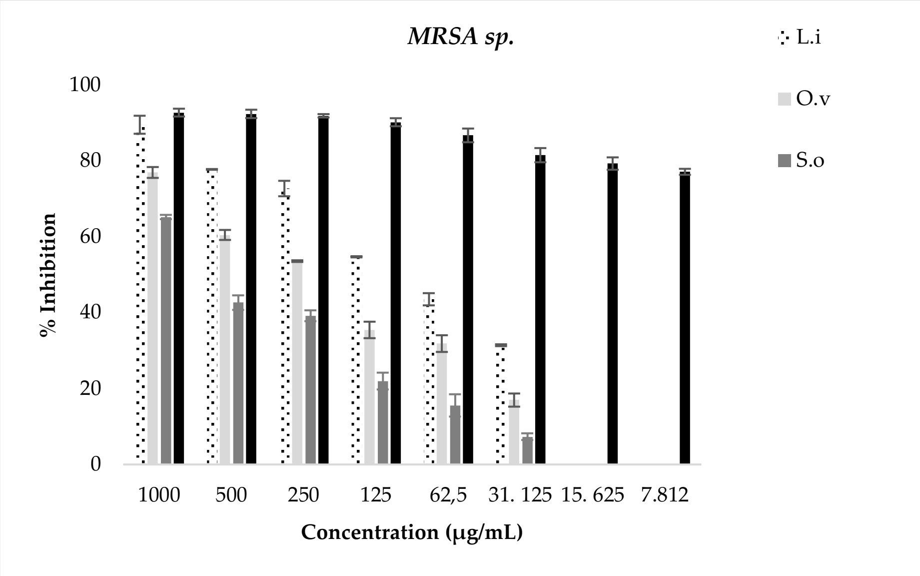

Figure 6. Inhibitory effect of L.i, O.v, and S.o with respective concentrations towards MRSA sp., n=3, O.v: *Origanum vulgare* subsp. *hirtum*; S.o: *Salvia officinalis*, L.i: *Lavandula x intermedia*

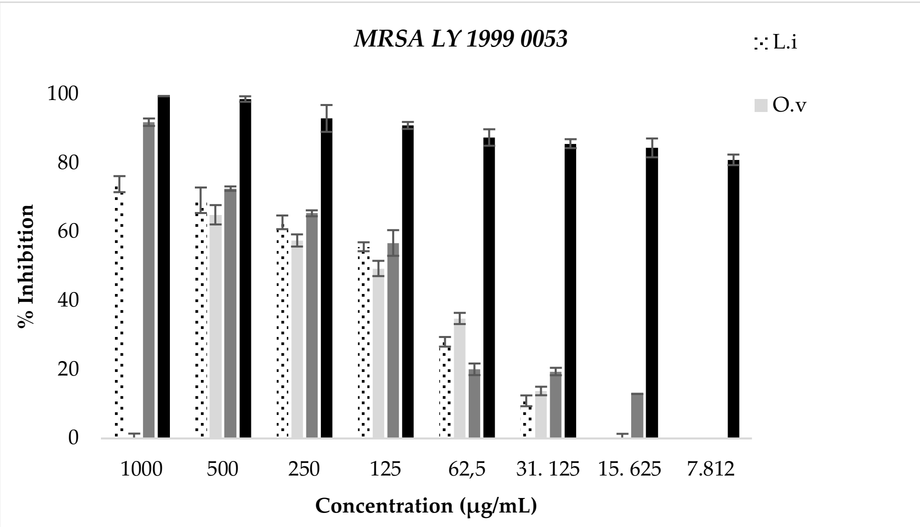

Figure 7. Inhibitory effect of L.i, O.v, and S.o with respective concentrations towards *MRSA* (LY 1999 0053), n=3, O.v: *Origanum vulgare* subsp. *hirtum*; S.o: *Salvia officinalis*, L.i: *Lavandula x intermedia*

This implies that sage EOs may have a wider range of activity against MRSA (LY 1999 0053) standard strain compared to other EOs. Ciprofloxacin demonstrated high inhibition at all concentrations, starting from 99.47%, and maintained this effect even at the lowest concentrations (Figure 7). This suggests that ciprofloxacin is a potent and broad-spectrum antimicrobial agent against MRSA (LY 1999 0053) standard strain.

The antibacterial activity of lavandin, oregano, and sage EOs on *Klebsiella* sp. was investigated. Lavandin EOs lost their activity at concentrations as low as 75.84% (at 1000 µg/mL), while oregano EOs were effective at moderate concentrations (up to 12.07%) starting from 90.77% (at 1000 µg/mL). Similarly, sage EOs decreased their activity starting from 84.45% (at 1000 µg/mL). These results suggest that all three EOs have antibacterial activity on *Klebsiella* sp., but the effect varies as the concentration decreases. Ciprofloxacin demonstrated strong antibacterial activity against *Klebsiella* sp., with a high initial inhibition rate of 99.11% that was maintained even at the lowest concentration of 7.812 µg/mL (86.92%) (Figure 8).

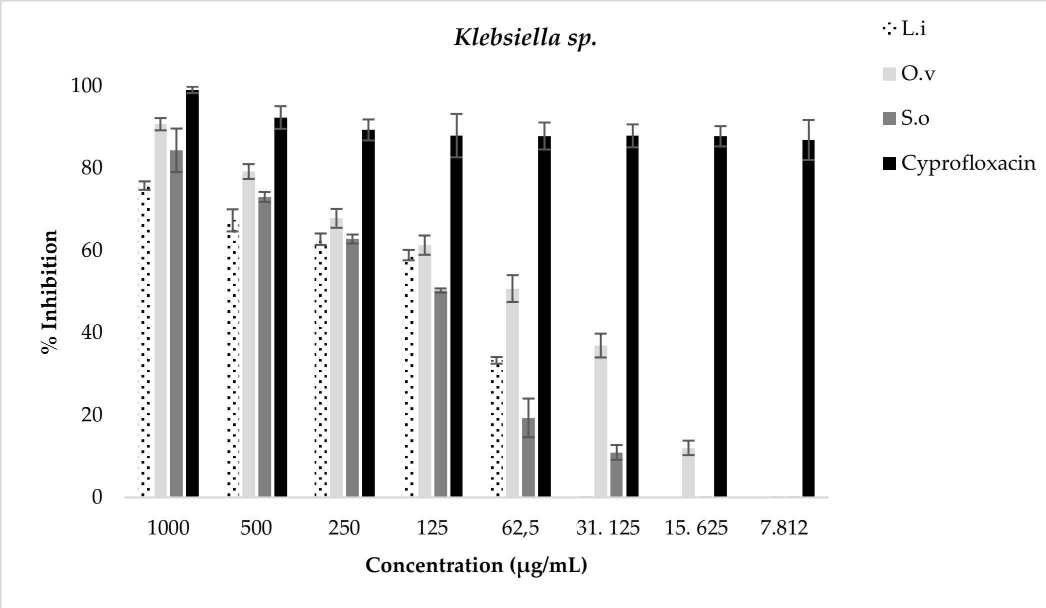

Figure 8. Inhibitory effect of L.i, O.v, and S.o with respective concentrations towards *Klebsiella sp.*, n=3, O.v: *Origanum vulgare* subsp. *hirtum*; S.o: *Salvia officinalis*, L.i: *Lavandula x intermedia*

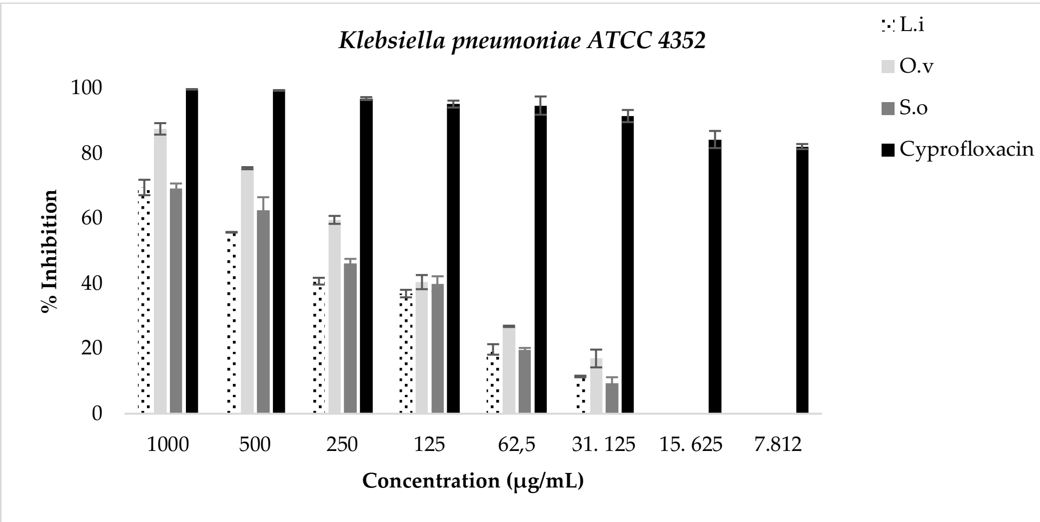

Figure 9. Inhibitory effect of L.i, O.v, and S.o with respective concentrations towards *Klebsiella pneumoniae ATTC 4352*, n=3. O.v: *Origanum vulgare* subsp. *hirtum*; S.o: *Salvia officinalis*, L.i: *Lavandula x intermedia*

At the highest concentration of 1000 µg/mL, lavandin EOs inhibited the growth of *Klebsiella pneumonia* ATCC 4352 standard strain significantly, with an inhibition rate of 69.59%. However, as the concentration decreased, the inhibitory effect also decreased, reaching a lower inhibition rate of 11.45% at 31.25 µg/mL. This suggests that the antibacterial activity of lavandin EOs is concentration-dependent. Among the EOs investigated, oregano EOs exhibited the strongest initial effect with an inhibition rate of 87.63% at 1000 µg/mL. However, this activity decreased as the concentration decreased and reached 16.98% at 31.25 µg/mL. These results suggest that oregano EOs is a highly effective antibacterial agent against *Klebsiella pneumonia* ATCC 4352 standard strain, although its activity decreases at low concentrations. At a concentration of 1000 µg/mL, sage EOs exhibited an inhibition rate of 69.26%, which decreased as the concentration decreased. At the lowest concentration tested (31.25 µg/mL), the antibacterial activity of sage EOs decreased significantly, with an inhibition rate of 9.34%. These results suggest that sage EOs are effective within a certain concentration range, but lose their effectiveness at lower concentrations. In the study, ciprofloxacin demonstrated the highest inhibition rates at all concentrations compared to the other substances analysed. The initial inhibition rate of ciprofloxacin was 99.79% at 1000 µg/mL, and even at the lowest concentration of 7.812 µg/mL, it exhibited an effective inhibition rate of 82.18% (Figure 9). The study shows that ciprofloxacin is a more effective antibacterial agent against *Klebsiella pneumoniae* ATCC 4352 standard strain than EOs, exhibiting consistent activity across a wide range of concentrations.

3.DISCUSSION

The chemical characterization results for *Origanum vulgare* subsp. *hirtum*, *Salvia officinalis*, and *Lavandula x intermediate* species are presented in detail. The EOs of these plants' main components are discussed, along with evaluations of the potential health benefits of these components. Carvacrol, the most dominant component of *Origanum vulgare* subsp. *hirtum* is responsible for the plant's antibacterial and antioxidant properties. Current research indicates that carvacrol has a potent inhibitory effect on various microorganisms and may have anti-inflammatory properties [33,39,40]. The presence of p-Cymene (5.51%) and 1,8-Cineole (2.13%) in the plant suggest potential health benefits, particularly for respiratory disorders [41]. *Salvia officinalis* contains high amounts of *α*-thujone (17.472%), camphor (13.594%), and borneol (12.143%) components. These components may contribute to the plant's antibacterial and anti-inflammatory effects. Camphor and borneol can be used as painkillers and antiseptics [42]. Lavandin EOs contain high levels of linalool (41.10%), linalyl acetate (16.66%), and camphor (10.01%). These components are believed to offer a wide range of health benefits, with linalool and linalyl acetate being particularly known for their calming and anxiety-reducing effects. Additionally, camphor has analgesic and anti-inflammatory properties]. The chemical characterization results suggest that the studied EOs may have beneficial effects on overall health. Specifically, their antibacterial and anti-inflammatory properties make them potential candidates for use in alternative medicine. Existing literature supports the positive effects of these ingredients on health, but further research is needed to determine their exact mechanisms and applications [43,44].

The study evaluated the antioxidant capacities of oregano, sage, and lavandin EOs using DPPH and ABTS tests. The tests measure the effectiveness of the EOs in neutralizing free radicals, providing information about their potential health benefits. The results showed that oregano EOs had an antioxidant capacity of 97.20% at a concentration of 50 µg/mL, while sage had 88.42% and lavandin had 76.72%. The results indicate that plant EOs are highly effective against free radicals. Oregano EOs, in particular, exhibit the highest antioxidant activity. Similar studies using the same methodologies confirm the generally high antioxidant capacities of these plants. For instance, one study observed that oregano and sage EOs have high antioxidant activity, which is attributed to the phenolic components of the EOs. Lavandin EO has demonstrated high antioxidant activity, with components such as linalool and linalyl acetate playing a significant role in this effect [45,46].

The ABTS test assesses the ability of antioxidants to neutralize particular free radicals, such as hydrogen peroxide. Our study's data indicates that these EOs have high ABTS radical scavenging activities. Oregano EOs, in particular, exhibited a potent antioxidant effect, particularly at high concentrations. Similar results are supported by other studies in the current literature that use the ABTS test [47,48]. Oregano, sage, and lavandin EOs exhibit potent antioxidant activity, primarily due to their high phenolic concentrations. Haddou et al. [49] investigated the pharmacological activities of *Lavandula pinnata* EOs (LPEO), including its antioxidant, antimicrobial, anti-inflammatory, and potential anti-cancer effects. These findings support the traditional use of LPEO and suggest potential for broader biological activities.

Another study has shown that the application of 1-MCP and lavandin EOs significantly improves the cold tolerance and quality characteristics of papaya fruit. Specifically, it has been determined that lavandin EOs reduce oxidative damage during cold stress by increasing antioxidant enzyme activity. These findings suggest that lavandin EOs could be a natural solution for preserving and extending the shelf life of agricultural products [50]. Yarnia et al. [51] found that biofertilizers and varying phosphorus levels had a positive impact on the biochemical properties, EOs content, and antioxidant enzyme activities of *Lavandula angustifolia*. These treatments resulted in an increase in EOs yield and a strengthening of the plant's antioxidant defence systems.

A comparative analysis of EOs from *Lavandula abrialis* and *Lavandula stoechas* showed that both species exhibit strong antioxidant and antimicrobial activities. These effects have been attributed to phenolic compounds and terpenes found in the chemical compositions of EOs [52]. The studies suggest that lavandin EOs and its components have potential applications in health and agriculture due to their antioxidant, antimicrobial, and anticancer properties. This makes lavandin EOs an attractive option for use as a natural therapeutic agent.

Walasek-Janusz et al. [53] investigated the antioxidant activities of oregano EOs obtained from Türkiye, USA, Poland, and Europe using DPPH as the free radical source. The study found that the values of antioxidant activities obtained were in the range of 71.42–80.44% and the oregano EOs obtained from Türkiye are the lowest. Baydır et al. [54] found *Salvia officinalis*'s antioxidant activity as 76.91%. Our result is higher than this study. de Torre et al. [55] studied *Origanum vulgare* subsp. *vulgare* and the antioxidant activity is measured against two radicals, DPPH and ABTS. In both assays, the oregano extract shows high activity. Machado et al. [56] assessed the impact of sage EOs on the growth, oxidative stress, and inflammatory response of calves. Sage EOs found to have potent antioxidant effects, which could enhance the oxidative stress response of animals. Aebisher et al. [57] studied the antioxidant activity of EOs from Seven Lamiaceae Plants including oregano, sage, and Lavandula with DPPH and ABTS assays. The results showed the studied EOs had a significantly stronger scavenging activity against the ABTS radical than against the DPPH. Despite this, they should be considered confirmation of the DPPH assay. Additionally, the antioxidant activity relation of EOS described as: oregano >sage >lavandula. In a study, ABTS and DPPH experiments, lavandin species had variable levels of bioactive components and antioxidant activity. The antioxidant activities of different lavandin species were investigated using the DPPH and ABTS methods and were found to be in the range of 66.56%-94.65% and 9.98-14.07 µgEqT/mL [58]. The antifungal activity of tea tree, thyme, cinnamon, and sage EOs was investigated in a study. Chemical analyses were conducted on thyme and other EOs to investigate their antioxidant and antifungal properties. Thyme EOs, in particular, demonstrated significant antifungal effects when used alone or in combination against selected Fusarium species [59]. Our study is in line with the existing literature and demonstrates that oregano EOs possess strong antioxidant and antibacterial properties due to their ability to neutralize free radicals and inhibit microorganisms. These properties make thyme EOs a promising candidate for use in the food preservation, healthcare, and cosmetic industries.

Sánchez-Vioque et al. [60] investigated the biological properties of by-products obtained from the distillation of EOs of Spanish sage (*Salvia lavandulifolia* Vahl.). The authors evaluated the by-products for their antioxidant, chelating, and antimicrobial activities. The results indicate that sage by-products exhibit potent antioxidant and antimicrobial activities. A study investigated the chemical and biological antioxidant activities of Clary sage (*Salvia sclarea*) EOs. The research suggests that clary sage EOs has potential as an antioxidant and anticancer agent. It is emphasized that short-term exposure to sage EOs is important for optimal antioxidant and anticancer effects [61]. These studies demonstrate that sage, particularly the EOs of the clary sage species, possess potent antioxidant properties. These properties may have potential uses in treating various health conditions. Furthermore, the assessment of sage by-products is an important approach that can contribute to the sustainability of herbal EOs production processes. These findings suggest that sage EOs and their byproducts could be valuable sources of antioxidants in the food, health, and cosmetic industries. It is recommended that further investigation be conducted to explore this potential. Thyme, sage, and lavandin EOs exhibit high antioxidant capacity, as indicated by the DPPH and ABTS results. Current research supports these findings and suggests that plant EOs may have beneficial effects on health.

EOs possess antioxidant properties that can reduce oxidative stress in cells by decreasing reactive oxygen species (ROS), thereby limiting the growth of cancer cells. The study presents cytotoxicity results against HeLa cells, demonstrating the effects of thyme, sage, and lavandin EOs at varying concentrations on cell growth. Higher concentrations resulted in increased cytotoxicity values. For instance, at 50 µg/mL, thyme EOs exhibited 35.58% cytotoxicity, and similar effects were observed for sage and lavandin EOs at similar concentrations. These results suggest that plant EOs have dose-dependent cytotoxic effects and may inhibit cell growth at certain concentrations. The current research on the anticancer effects of lavandin EOs is very promising. Han et al. [62] demonstrated the anticancer effects of α-pinene, a natural terpene found in the EOs of various plants including *Lavandula angustifolia*, on gastric cancer cells. The research suggests that α-pinene could be a potential cancer treatment agent by inducing cell death and inhibiting the proliferation of cancer cells. α-pinene is a natural terpene found in the EOs of various plants, including *Lavandula angustifolia* and *Satureja myrtifolia*. According to this study, α-pinene has antioxidant, antibiotic, and anticancer properties. Although the study did not specifically focus on lavandin EOs, it did examine the anticancer effects of α-pinene against AGS gastric cancer cells. The study examines the biological activity of *Lavandula pinnata* EOs (LPEO), which is derived from a lavandin species indigenous to the Canary Islands and has been traditionally used in various treatments. Research has demonstrated that LPEO exhibits several pharmacological activities, including anticancer effects [49].

Sharma et al. [63] evaluated the effects of EOs constituents on steroidogenic cytochrome P450 activity and suggested that EOs such as lavandin EOs or their metabolites may have potential therapeutic effects against cancer cells. The research indicates that lavandin EOs and other EOs could provide further information about the mechanism of their anticancer properties. According to these studies, lavandin EOs and their components may have the potential to fight cancer. However, further research is required to fully comprehend the mechanisms behind lavandin EO's anticancer effects and to establish whether these effects have clinical significance. Although these studies suggest that lavandin EOs may have potential in cancer treatment, clinical studies evaluating their effectiveness and safety in humans are necessary.

Hashem et al. [64] studied the characterization and biological activities of a new nanoemulsion based on clove and thyme EOs in their nanoemulsion study using thyme EOs. The anticancer activity of the nanoemulsion was evaluated on various cancer cell lines, and the findings suggest that thyme EOs may be a potential tool in fighting cancer. A study has indicated that the EOs derived from the aerial parts of *Thymus persicus* are a valuable natural source of anticancer triterpenic acids (TAs). It has been reported that the EOs are obtained at a rate of 0.35%, and similar rates are found in thyme species endemic to Türkiye. The potential anticancer properties of these triterpenic acids support the use of thyme EOs against cancer [65]. The study evaluated the potential therapeutic effects of *Thymus vulgaris* L. EOs (thyme EOs) against human breast cancer cell lines, both in silico (computer simulation) and in vitro (laboratory experiments). The cytotoxicity and antiproliferative activity of oregano EOs against breast cancer cell lines has been confirmed, indicating its potential as an anticancer agent [66].These studies are significant in exploring the effects of thyme EOs and its components on cancer cells and their potential therapeutic uses. Further research is required to understand the exact mechanisms of thyme EO's anticancer effects and to evaluate its safety and effectiveness for clinical applications.

Falih et al. [67] suggest that clary sage (*Salvia sclarea*) EOs may have antioxidant and anticancer properties. Research indicates that short-term exposure to sage EOs is crucial for achieving optimal antioxidant and anticancer effects. These findings suggest that sage EOs could be a potential tool in the fight against cancer. This paper reviews the potential health benefits of medicinal sage (*Salvia officinalis*), including its anti-cancer and anti-inflammatory properties. It is suggested that products containing sage EOs may have potential in the fight against cancer [68]. These studies are significant in exploring the effects of sage EOs and their components on cancer cells and their potential therapeutic uses. Further research is required to understand the exact mechanisms of sage EOs's anticancer effects and to evaluate their safety and effectiveness for clinical applications.

The cytotoxicity data obtained in our study support the potential inhibitory effects of thyme and sage EOs, particularly on cancer cell lines, when compared with findings in current articles. For instance, in silico and in vitro studies have evaluated the anticancer potential of *Thymus vulgaris* EOs against breast cancer cell lines, confirming its anticancer potential through cytotoxicity and antiproliferative activity. Similarly, it has been noted that clary sage (*Salvia sclarea*) EOs can act as an antioxidant and anticancer agent. Optimal antioxidant and anticancer effects require short-term exposure intervals. The cytotoxicity results in the study are in harmony with the studies in the current literature. Both sources suggest that plant EOs have potential cytotoxic effects that may inhibit cell growth at certain concentrations. They may have potential as adjunctive therapeutic agents in the treatment of cancer. However, further research is needed to understand the exact mechanisms of these effects and to evaluate their safety and effectiveness for clinical applications.

The study evaluated the antibacterial activities of lavandin, oregano, and sage EOs against different clinical isolates and standard bacterial strains. Lavandin EOs exhibited a high inhibition rate of 90.77% at a concentration of 1000 µg/mL, while oregano EOs showed a strong antibacterial effect at all concentration levels. At a concentration of 1000 µg/mL, sage EOs exhibited an inhibition rate of 82.45%. However, this effect decreased significantly as the concentration decreased. Abd Ellah et al. [69] investigated the antibacterial activity of vaginal gel formulations containing tea trees and lavender EOs. The study found that the combination of these EOs exhibited moderate antibacterial activity, particularly against *Candida albicans*. The optimal gel-forming and EOs concentrations were crucial in determining the antibacterial effectiveness of the formulation.

Loh et al. [50] performed a study that showed the lavender EOs were effective in improving the quality characteristics and cold tolerance of papaya fruit exposed to cold stress. The antioxidant and antimicrobial properties of lavender EOs are crucial in extending the shelf life of the fruit and reducing damage caused by cold stress. Mourabiti et al. [70] carried out a comparison of the antimicrobial activities of three different EOs (rosemary, lavender, and sage) and found that lavender EOs in particular had a strong antimicrobial effect against gram-negative bacteria such as *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The chemical composition of the EOs was directly linked to its antimicrobial activity.

Sadeghi et al. [71] found that nanofibers created by combining lavender EOs with gelatin biopolymer exhibited significant antimicrobial activity against bacteria, including *Staphylococcus aureus* and *Escherichia coli*. These nanofibers have been assessed as a potential antimicrobial surface material for treating wounds. The studies suggest that lavender EOs possess effective antimicrobial properties against various microorganisms. These properties may have potential applications in healthcare, food preservation, and biomedical fields.

Zhou et al. [72] developed slow-release packaging systems containing thyme EOs to extend the shelf life of fruits and vegetables. The moisture sensitivity of the packaging optimizes the antimicrobial properties of the EOs according to environmental conditions. As humidity increases, the release of antimicrobial substances increases, improving the preservation of stored food products. The study shows that thyme EOs have the ability to inhibit microbial growth, and this property can be utilized in packaging technologies. The use of Gram-negative rough mutants allows more sensitive detection of the antimicrobial activity of EOs in situations where EOs, such as thyme EOs, may have an advantage over synthetic antimicrobial drugs. This is especially important in determining the effectiveness of EOs on challenging microorganisms [73]

Dallal et al. [74] investigated the potential treatment effects of thyme EOs and endothelial progenitor stem cells (EPCs) on lipopolysaccharide-induced sepsis. Thyme EOs exhibited antimicrobial and anti-inflammatory effects on mice in a sepsis model. These findings indicate that the EOs and EPCs may have potential for use in sepsis treatment. These results suggest that thyme EOs may be effective against a wide range of microorganisms and may be used in various applications, particularly in preserving foods and in medical treatments. Further research is required to gain a better understanding of the mechanisms behind the antimicrobial effects of thyme EOs and their practical applications.

Šarić et al. [75] investigated the antimicrobial properties of EOs from sage (*Salvia officinalis*), rosemary (*Rosmarinus officinalis*), and lavender (*Lavandula angustifolia*) against *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The results showed a significant antibacterial effect of these EOs, particularly against *Pseudomonas aeruginosa*. EOs are known to contain compounds that can inhibit the growth of microorganisms. The evidence indicates that these EOs have potential as antimicrobial agents, particularly against gram-negative bacteria. This activity is likely due to the presence of antimicrobial components in EOs, such as phenolic compounds and terpenes.

A study evaluated lozenge formulations containing sage EOs as a potential alternative for oral care. The study focused on the antimicrobial properties of sage EOs and found that lozenges containing sage EOs were effective against oral pathogens. These findings suggest that sage EOs may be a natural alternative for maintaining oral and dental health, potentially preventing plaque formation, and helping to maintain intraoral microbial balance [76].

Atmaca et al. [77] investigated the antimicrobial and antifungal properties of creams enriched with EOs of various medicinal aromatic plants. The use of objective language and precise terminology ensured that the results were conveyed accurately. It was found that sage EOs exhibited significant effects against certain types of bacteria and fungi that cause skin infections. The study demonstrates that sage EOs have potential as a natural protective and therapeutic agent in skin care products due to their antimicrobial effects. This suggests that sage EOs could be beneficial for skin health applications. The data suggest that sage EOs may be effective against a wide range of microorganisms and that these EOs may have potential uses in a variety of applications, particularly in food preservation, healthcare, and cosmetics. Further research is required to fully comprehend the exact mechanisms of its antimicrobial effects. The findings on antimicrobial activity presented in this study show that EOs have different antimicrobial activities depending on their concentrations. This underscores that EOs can be effective against specific microorganisms, but optimal antimicrobial activity requires determining appropriate concentrations. Certain plant EOs, such as thyme and sage, seem to exhibit antimicrobial effects at particular concentrations, but these effects may diminish as the concentration decreases. These findings suggest that EOs could be used as alternative or supportive agents in the treatment of microbial infections.

4.CONCLUSION

This study examines the bioactive properties of EOs from *Salvia officinalis*, *Origanum vulgare* subsp. *hirtum*, and *Lavandula x intermedia* (Lavandin). The research focused on the antioxidant, antibacterial, and cytotoxic activities of the EOs of these plants. The study provided significant findings about their potential therapeutic applications and positive effects on health. Antibacterial tests revealed that oregano EOs exhibit a strong effect over a wide concentration range, while lavandin and sage EOs are also effective at certain concentrations. Antioxidant activity evaluations have demonstrated that the radical scavenging abilities of all herbal EOs differ depending on concentration. Cytotoxicity results indicate their potential to inhibit cell growth at high concentrations, suggesting that these EOs may be possible candidates for use in cancer research.

In conclusion, this study has demonstrated that *Salvia officinalis* (CV Beyhekim), *Origanum vulgare* subsp. *hirtum* (CV Uluğ Bey), and *Lavandula x intermedia* possess significant antibacterial, antioxidant, and cytotoxic properties. These findings suggest that these plant EOs may have potential applications in the management and treatment of various health problems. The results of this study provide a foundation for further research into the pharmacological uses of plant EOs. However, further clinical studies are required to gain a better understanding of the effectiveness and safety profiles of these EOs. This is essential to enable the more effective and safe use of plant EOs in the health sector.

5. MATERIALS AND METHODS

5.1. Materials

5.1.1. Certain EOs Physicochemical Parameters of the Experimental Site

The plantations were established in the Experimental and Production Center of the Field Crops Central Research Institute, Gölbaşı İkizce, Ankara located at 39613319N and 32671541E. The EOs samples were taken in the plantation area at a depth of 0-30 cm on July 17th, 2022, and sent to be analyzed at the EOs Fertilizer and Water Resources Institute, Ankara. The EOs had a loam/clay-loam texture and the EOs pH was determined slightly alkaline (7.60-7.74). The lime ratio was found in high categorized (29.8-30.3) in the heavy-lime group. The organic matter rate was 0.95-1.92% law which was categorized in the insufficient group. The electrical conductivity (EC) of the EOs was 0.64 ds/m salt-free (0-2 salt-free). The available Phosphorus (P_2O_5) amount was determined as 48 kg/ha (insufficient) and potassium (K) as 1560 kg/ha. The microelement analysis of the EOs was accordingly; 3.43 ppm available iron, Fe (0.2-4.5 medium), 1.35 ppm available copper (Cu) (>0.2 sufficient), 0.27 ppm available Zinc, and 3.21 ppm available Manganese (Mn) which are categorized in very low concentrates [79,80].

5.1.2. Plant-Materials

The Oregano (CV Uluğ Bey) was harvested on July 22nd2022, from three years old plantations, at the 50% flowering stage, when the EOs ratio was the highest amount in Central Anatolian climatic conditions [81]. Common sage plants were provided from a four years old plantation, 'CV Beyhekim', which were harvested on 6 June 2022. The herb samples of oregano and common sage were dried for three days in a drying cabinet at 35 ºC. Their EOs compositions are also shown in Table 1, and Table 2. The EOs of *Lavandula* x *intermedia* used in the present research as plant material was harvested on July 14, 2022, and its chemical components are given in detail in Table 3.

5.2. Methods

5.2.1 Isolation of the EOs

Hydrodistillation, method was used to derive EOs and bioactive compounds from plant materials. EOs were extracted by hydrodistillation from 100 g seeds and dry leaf and flower herb materials using a Neo-Clevenger apparatus for three hours, in the Medicinal and Aromatic Plants Unit Laboratory of CRIFC, Ankara, Türkiye. A Chillers (Buchi F-314) apparatus was connected to the Neo-Clevenger to set the central temperature and cool the system for a better and more sustainable EOs formation. The main advantage of this extraction technique is its ability to isolate plant materials below 100°C [82]. Neo-Clevenger was cleaned by running the system empty before each analysis. The extracted EOs were kept in amber vials at +4°C until they were identified by GC-MS.

5.2.2. Determination of EOs Components for Origanum and Common Sage

An Agilent 5975 GC-MSD system was used for GC/MS analysis. Helium carrier gas (0.8 mL/min) was used on an Innowax FSC column (60 m x 0.25 mm, 0.25 mm film thickness). Essential oil (EO) samples were distilled 1:100 with hexane and injected into the column (0.2 µL) at a 40:1 split ratio. The oven temperature was initially set at 60°C and raised at 4°C/min to 220°C, where it was kept for 10 min. The injector temperature was set to 250°C. The analysis time for each sample was 60 min. Electron bombardment ionization at 70 eV was used for the mass range m/z 35 to 450. EO components were identified by comparing their mass spectra with those in the Adams, Wiley GC/MS, and Mass Finder libraries and confirmed by comparing relative retention times and relative retention indices (RRI) [83-85].

5.2.3. Determination of 1,1-Diphenyl-2-picrylhydrazil (DPPH•) Radical Scavenging Effect

The DPPH• free radical reduction effects of the essential oils (EOs) were determined using the method described by Gyamfi et al. [86]. For the DPPH analysis, triplicate dilutions of 1:32, 1:16, 1:4, 1:2, and 1:1 were prepared for each EO. The EOs were diluted with 20% dimethylsulfoxide (DMSO). The DPPH solution was prepared using Tris-HCl buffer (50 nM, pH 7.4) and 0.1 mM of methanol. The control solution was prepared by mixing 50 µL of methanol and 2 mL of a 6×10^{-5} methanolic DPPH solution. Absorbances at 517 nm were measured following a 30-minute incubation period at 25 ◦C in the dark. The following formula was used to determine the % inhibition values:

$$Inhibition\ (\%) = \frac{Abscontrol - Abssample}{Abscontrol}\ x\ 100$$

where Abscontrol is the absorbance of the control (DPPH solution plus methanol) and Abssample is the absorbance of the sample including DPPH plus the EOs.

5.2.4. ABTS•+ Radical Scavenging Activity

The ABTS•+ radical scavenging activity of s EOs was determined following the method described in the literature [87]. An aqueous solution of ABTS and K2S2O8 (2.45 mM, final concentration) was kept in the dark for 12-16 h to obtain ABTS•+ (7 mM). Absorbance was calibrated at 734nm to 0.700 (±0.030). The kinetics of the reaction was determined and measured at 734 nm at 1-minute intervals for 30 min using 990 µL of the generated radical solution and 10 µL of the EOs samples. The percent inhibition versus concentration (TEAC) of Trolox equivalents was evaluated. As positive control, BHT was added. Experiments were performed three times and averages were calculated.

5.2.5. Cell Culture

Hela cell lines were supplied from the American Type Culture Collection (CCL222; CCL-2). The cells were cultured in RPMI and DMEM, respectively, with 1% double antibiotics (penicillin and streptomycin) and 10% fetal bovine serum at 37°C and 5% CO2.

Cytotoxic Activity

Hela cells were seeded in a 96-well plate at a density of 1×104 cells/mL 100 (100 µl per well) and divided into blank, control, and extracts (7.81; 15.6; 31.25; 62.5; 125; 250; 500, and 1000 µg/l) groups. After incubation for 24 hours, cells were treated with 100 µl of vehicle or samples for 24 hours. Next, MTT reagent (stock: 5 mg/ml in PBS) was added into each well and incubated at 37°C for 4 hours. Each well received 100 µl of DMSO, which was used to dissolve the formazan crystals generated by MTT. Using a microplate reader with a 540 nm wavelength, each well was read after 10 minutes

5.2.6 Antibacterial effects

Preparation of EOs dilutions

EOs dilutions were prepared by dissolving 320 mg of pure EOs in 1280 µL of Dimethyl Sulfoxide (DMSO). This solution was further diluted by adding 400 µL to achieve a concentration of 80 mg/mL. Subsequent dilutions were prepared in equal proportions from this benchmark concentration, resulting in concentrations ranging from 80 mg/mL to 0.03 mg/mL.

Bacterial culture

The antibacterial activity of the EOs was tested against standard bacterial strains of *Acinetobacter baumannii* ATCC 17606, Methicillin-Resistant *Staphylococcus aureus* (MRSA) LY 1999 0053, and *Klebsiella pneumoniae* ATCC 4352, including *Acinetobacter* sp., *Klebsiella* sp. and MRSA sp. obtained from reputable sources. The strains were cultured following the Clinical and Laboratory Standards Institute (CLSI) guidelines to ensure standardized growth conditions. The detection of Minimum Inhibitory Concentration (MIC) was carried out by inoculating microorganisms into Cation Adjusted Muller Hinton Broth (CAMHB) in microplates. The antibacterial effects were evaluated using spectrophotometry.

The Minimum Inhibitory Concentration (MIC)

The detection of Minimum Inhibitory Concentration (MIC) involved inoculating the cultured microorganisms into Cation Adjusted Muller Hinton Broth (CAMHB) in U-bottomed, sterile, capped microplates. The wells were then filled with the EOs, antibiotics, and their combinations in descending concentration order. Following the incubation period, the absorbance of the microplates was measured at 625 nm using a spectrophotometer. The antibacterial efficacy of the EOs could not be determined through MIC and MBC analyses using the microdilution method as its MIC values exceeded the measurement range.

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