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Combinations of essential oils obtained from medicinal plants with *L. angustifolia*: Determination of their antimicrobial effects

Tıbbi bitkilerden elde edilen uçucu yağların *L. angustifolia* ile kombinasyonları: Antimikrobiyal etkilerinin belirlenmesi

Derya Doğanay[1*], Şevval Maral Özcan-Aykol[2], Vildan Seyhan[3], İlker Demirbolat[4], Esra Mertoğlu[5]

[1]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Health Sciences, İstanbul, Türkiye

[2]Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Biruni University, İstanbul, Türkiye

[3]Independent Pharmacist, Seyhan Pharmacy, İstanbul, Türkiye

[4]Department of Pharmaceutical Chemistry, Faculty of Pharmacy, İstanbul Kent University, İstanbul, Türkiye

[5]Department of Phytotherapy, Hamidiye Institute of Health Sciences, University of Health Sciences, İstanbul, Türkiye

***Correspondence:**
ddoganay2904@gmail.com

ORCID iD:
0000-0001-9147-4110

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ABSTRACT

In this study, the antimicrobial effects of essential oils obtained from 7 different plants (*Lavandula angustifolia*, *Lavandula intermedia*, *Cupressus sempervirens*, *Syzygium aromaticum*, *Salvia officinalis*, *Allium sativum*, *Pimpinella anisum*) and their combinations with *Lavandula angustifolia*, were investigated against 11 pathogenic bacteria (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus haemolyticus* ATCC 43252, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC NRRLB 3704, *Acinetobacter baumannii* ATCC 19606, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 13315, *Enterococcus faecalis* ATCC 29212, *Aeromonas hydrophila* ATCC 95080). The synergistic effect of essential oils and their combinations with *L. angustifolia* were determined using the agar well diffusion method. Each essential oil was subjected to gas chromatography analysis in order to identify their chemical compositions. According to the results, generally all essential oils exhibited the greatest inhibition zones for Gram-positive bacteria than for Gram-negative bacteria. Among the essential oil combinations, the highest synergistic effect (166.7%) was obtained in *L. angustifolia-P.anisum* combination against *P. aeruginosa* ATCC 27853. Additionally, the *L. angustifolia-S. aromaticum* combination was observed to exhibit a synergistic effect on 5 bacteria (*K. pneumoniae*, *E. coli*, *E. faecalis*, *S. haemolyticus* and *A. hydrophila*). These findings suggest that *L. angustifolia* combined with specific essential oils particularly *L. intermedia* and *S. aromaticum* may serve as promising natural antimicrobial agents, offering enhanced inhibitory activity against clinically important bacterial pathogens.

Keywords: Essential oils, antibacterial activity, agar well diffusion method, synergistic effect, pathogen bacteria, inhibition zone

ÖZET

Bu çalışmada 7 farklı bitkiye (*Lavandula angustifolia*, *Lavandula intermedia*, *Cupressus sempervirens*, *Syzygium aromaticum*, *Salvia officinalis*, *Allium sativum*, *Pimpinella anisum*) ait uçucu yağın ve bunların *Lavandula angustifolia* ile kombinasyonlarının 11 patojen bakteriye (*Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus haemolyticus* ATCC 43252, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC NRRLB 3704, *Acinetobacter baumannii* ATCC 19606, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 13315, *Enterococcus faecalis* ATCC 29212, *Aeromonas hydrophila* ATCC 95080) karşı antimikrobiyal etkileri araştırılmıştır. Uçucu yağların ve *L. angustifolia* ile kombinasyonlarının sinerjik etkisi agar kuyu difüzyon yöntemi ile belirlenmiştir. Her bir uçucu yağ, kimyasal bileşimlerini belirlemek amacıyla gaz kromatografisi analizine tabi tutulmuştur. Sonuçlara göre genel olarak test edilen tüm uçucu yağlar Gram-pozitif bakteriler için Gram-negatif bakterilerden daha büyük inhibisyon zonu göstermiştir. Uçucu yağ kombinasyonları arasında en yüksek sinerjik etki (166,7%) *Pseudomonas aeruginosa* ATCC 27853'e karşı *L. angustifolia-P. anisum* kombinasyonunda elde edilmiştir. Ayrıca *L. angustifolia-S. aromaticum* kombinasyonunun 5 bakteri (*K. pneumoniae*, *E. coli*, *E. faecalis*, *S. haemolyticus* ve *A. hydrophila*) üzerinde sinerjik etki gösterdiği gözlenmiştir. Bu bulgular, *L. angustifolia*'nın belirli uçucu yağlarla (özellikle *L. intermedia* ve *S. aromaticum*) birlikte kullanıldığında, klinik açıdan önemli bakteriyel patojenlere karşı gelişmiş inhibitör aktivite sunarak umut verici doğal antimikrobiyal ajanlar olarak işlev görebileceğini düşündürmektedir.

Anahtar kelimeler: Uçucu yağlar, antibakteriyel aktivite, agar kuyu difüzyon yöntemi, sinerjik etki, patojen bakteri, inhibisyon bölgesi

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Essential oils are volatile secondary metabolites of plants and complex mixtures of natural aromatic compounds. They can be isolated through physical processes like steam or hydrodistillation. They are widely used for their various abilities in cosmetics, aromatherapy and medicine (Sarkic & Stappen, 2018). Likewise, they are indispensable for the fragrance industry. Essential oils contain a wide variety of volatile compounds such as terpenes and terpenoids, phenol-derived aromatics and aliphatic components.

As they are primarily used for their scents in fragrance industry, they have beneficial anti-inflammatory, antibacterial, digestion stimulant and decongestant activities. They can be used in type 2 diabetes, cardiovascular diseases, osteoporosis and for the prevention and treatment of cancer (Demirbolat et al., 2019; Tisserand & Young, 2014). One of the most common utilizations of essential oils depends on their antimicrobial activities. Various essential oils like lavender (*Lavandula angustifolia* Mill.), lemon balm (*Melissa officinalis* L.), juniper berry (*Juniperus communis* L.), lemon verbena (*Lippia citriodora* Kunth), rosemary (*Rosmarinus officinalis* L.) and cypress (*Cupressus sempervirens* L.) were investigated earlier for their antimicrobial activities (Garzoli et al., 2019; Jianu et al., 2013; Mazari et al., 2010; Saricaoglu & Turhan, 2018; Sienkiewicz et al., 2014). Composition of essential oils vary between species, harvesting conditions, extraction techniques and even within the genders of the same species (Demirbolat et al., 2020). Therefore, the outcome of the antimicrobial studies might also be altered according to the essential oil compositions and the strains that were used for a particular study.

In this study, 7 different essential oils and 11 different pathogens were utilized for antimicrobial activities. All tested essential oils were analyzed in order to determine their chemical compositions. Bioactivities of single essential oils and mixtures of some selected ones were evaluated by determining their inhibitory zones.

Materials and methods

Essential oils

Lavander (*Lavandula angustifolia*), lavandin (*Lavandula intermedia* Emeric ex Loisel) cypress (*Cupressus sempervirens*), clove bud (*Syzygium aromaticum*), sage (*Salvia officinalis*), garlic (*Allium sativum*) and anise (*Pimpinella anisum*) essential oils and some of their 1:1 combines (*Lavandula angustifolia-Lavandula intermedia*, *Lavandula angustifolia-Cupressus sempervirens*, *Lavandula angustifolia-Syzygium aromaticum*, *Lavandula angustifolia-Salvia officinalis*, *Lavandula angustifolia-Allium sativum*, *Lavandula angustifolia-Pimpinella anisum*) were used for antibacterial activity tests. All essential oils were obtained from the Phytotherapy Research Center of Bezmialem University.

Essential oil analysis

Solutions of 10% (v/v) essential oils in *n*-hexane were subjected to GC-FID/MS analysis. An Agilent7890B GC-FID (Santa Clara, CA, USA) coupled with an Agilent 5977E electron impact mass spectrometer (Santa Clara, CA, USA) via a two-way capillary splitter was utilized to identify and quantify essential oil components. An Agilent G45-13A (Santa Clara, CA, USA) auto-injector was employed for 1 µL sample injections. DB-WAX column (60 m, 0.25 mm, 0.25 µm) was operated with the following temperature program; 700 °C at 15 min and raised to 1800 °C at a rate of 20 °C/min. 5 min isothermal at 1800 °C following a 50 °C/min rate to 230 °C and finally 15 min isothermal at 230 °C. Helium was used as a carrier gas with a constant flow of 1.5 mL/min. The split ratio was set to 50:1. Temperatures of injector port, ion source, quadrupole, MSD transfer line and FID were as follows respectively; 220 °C, 230 °C, 150 °C, 250 °C and 220 °C. FID air flow was 400 mL/min and H_2 flow was adjusted to 30 mL/min. The mass detector scan range was set to 45-450 m/z.

Compounds were identified by comparing their spectral data obtained from the Wiley Registry of Mass Spectral Data 9th edition (April 2011) with NIST 11 Mass Spectral Library (NIST11/2011/EPA/NIH) and retention indices which were calculated from the co-injected alkane series (C7-C40) and derived from previous studies (Goodner, 2008). Quantitation of essential oils were performed via FID responses. All analyses were performed in triplicate.

Test microorganisms

The effect of essential oils was tested against the following bacteria: *Staphylococcus aureus* ATCC 25923, *Staphylococcus aureus* ATCC 29213, *Staphylococcus haemolyticus* ATCC 43252, *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Escherichia coli* ATCC NRRLB 3704, *Acinetobacter baumannii* ATCC 19606, *Bacillus subtilis* ATCC 6633, *Proteus vulgaris* ATCC 13315, *Enterococcus faecalis* ATCC

29212, *Aeromonas hydrophila* ATCC 95080. All strains were obtained from the culture collection at Biruni University, Faculty of Pharmacy, Department of Pharmaceutical Microbiology.

Preparation of test microorganisms

The bacteria stored at -80 °C used in the study were activated one day in advance on Mueller-Hinton Agar (MHA) medium at 37 °C for 24 hours. The bacterial were adjusted to 0.5 McFarland (108 CFU/ml) using Mueller Hinton Broth (MHB) medium and a McFarland device (BioSan) with the aid of a sterile pipette (CLSI, 2006).

Antibacterial activity

Determination of antimicrobial effects by agar well method

The agar well method was used for *in vitro* antimicrobial activity of essential oils against bacteria (CLSI, 2006; Magaldi et al., 2004; Valgas et al., 2007). Mueller-Hinton Agar (MHA) was used as the medium for bacteria. Particularly, a hole with a diameter of 6 to 8 mm was punched aseptically on Muller Hinton Agar plates with steril an agar drill. The prepared bacteria (100µL) suspensions were sown on the entire agar surface by spreading method. 20 µL of each essential oil was added to the agar wells inoculated with microorganisms. For positive controls 10 µg/mL streptomycin and 10 U penicillin was used. Sterile water or dimethyl sulfoxide (DMSO) was used as a negative control. All agar plates were incubated at 37 °C, 24 hours. At the end of the incubation, the circular diameter (inhibition zones) from the border of the well to the border where microorganism growth began was measured in mm with the help of calipers. The study were performed three times and the results were averaged.

The antimicrobial activity of L. angustifolia and other essential oil combinations

Determine antibacterial effects of essential oil mixtures, *L. angustifolia* essential oil was mixed with another essential oil in (1:1) concentration. An *L. angustifolia* mixture was preferred, since it was the most efficient antibacterial essential oil within all the tested oils. These combinations were named *L. angustifolia* and *L. intermedia* (A), *L. angustifolia* and *C. sempervirens* (B), *L. angustifolia* and *S. aromaticum* (C), *L. angustifolia* and *S. officinalis* (D), *L. angustifolia* and *P. anisum* (E), *L. angustifolia* and *A. sativum* (F). Agar well diffusion method was also used to determine the antibacterial activity of essential oil combinations. All experiments were conducted in triplicate. After incubation, the zones of inhibitions were measured and the fold increase in the diameter of inhibition zone of each *L. angustifolia* after combination with other oils was calculated according to the equation; The fold increase = (b-a)/a x100, Where; (a) is the inhibition zone of *L. angustifolia* alone and (b) is the inhibition zone of *L. angustifolia* in combination with other oils (Arsene et al., 2021, Fahmy et al., 2025). In addition to, the synergistic effects of essential oils combination were calculated using a web application based on the mathematical formula published by Arsène (2021).

Results and discussion

Chemical composition

Among the essential oils tested, *L. angustifolia* essential oil mainly contained linalyl acetate (23%) and linalool (22%). These compounds were followed by cis-ocimene (7%), lavandulyl acetate (5%) in less concentrations. *L. intermedia* essential oil contained mainly linalool (26%), linalyl acetate (20%), and in less concentrations 1,8-cineole (7%), camphor (6%). *S. officinalis* essential oil contained mainly β-thujone (20%), 1,8-cineole (16%), and camphor (15%), and in less concentrations α-thujone (9%), camphene (7%) and β-pinen (4%). *S. aromaticum* essential oil contained mainly eugenol (62%) and in less concentrations acetyleugenol (18%) and caryophyllene (17%). *C. sempervirens* essential oil contained mainly α-pinen (44%), and in less concentration β-pinen, Δ-3-carene (11%), and limonen (10%), *A. sativum* essential oil contained mainly diallyl disulphide (77%), and in less concentration diallyl sulphide (16%). *P. anisum* essential oil contained mainly *trans*-anethole (87%), and in less concentrations estragole (5%) and pseudoisoeugenol 2-methyl butyrate (4%). In Table 1, the first five compounds in the highest concentration of the essential oil tested are given. When the results were compared with the results previously provided by Lis-Balchin (2006), linalool and linalyl acetate concentrations of *L. angustifolia* essential oil, α-pinen concentration of *C. sempervirens* and *trans*-anethole, estragole concentration of *P. anisum* are well within the limits which were all the major compounds of the tested essential oil.

The main components of the essential oil of *L. angustifolia* are expected to be linalool 20-50%) and linalyl acetate (25-46%) and in less concentrations cis-ocimen (3-7%), terpinene-4-ol (3-5%), limonene, cineole, camphor, lavandulyl acetate, lavandulol and α-terpineol, β-caryophyllene, geraniol, α-pinen according to EMA

assessment report (EMA, 2012). For *L. angustifolia* the EMA report suggests that cis-ocimene should be present at lower concentrations, typically ranging from 3%-7%. In our analyzed oil, the cis-ocimene concentration was detected at 7%. This places the concentration at the upper limit of the expected EMA range. This characteristic, while not a dramatic deviation, distinguishes our sample's chemotype and may be attributed to specific regional, seasonal, or harvest-time variations. For *S. aromaticum* the EMA Monograph specifies that acetyl eugenol concentrations should typically be between 4% and 15%. Our analysis detected the acetyl eugenol concentration at 17%. This value is marginally higher than the upper limit specified by the EMA. This slight elevation in acetyl eugenol content confirms that while the oil is of high quality and generally compliant, its chemotype exhibits minor regional variation, likely due to differences in geographical origin, maturity of the plant material, or extraction process parameters. Eugenol (75–88%), acetyl eugenol (4–15%), β-caryophyllene (5–14%) are expected to be the major components of *S. aromaticum* essential oil according to EMA monograph. The tested essential oil composition of *S. aromaticum* is broadly in accordance with the EMA monograph, except for a slightly higher concentration of acetyl eugenol (eugenyl acetate), which was found to be 18.0% in our study, exceeding the monograph's upper limit of 15.0% (EMA, 2011). *S. officinalis* essential oil composition is also well in accordance with the references provided within EMA monograph (EMA, 2016).

Table 1. Major compounds of the analyzed essential oils

L. angustifolia	**C%**	***L. intermedia***	**C%**	***S. officinalis***	**C%**	***S. aromaticum***	**C%**
Linalyl acetate	23	Linalool	26	β-thujone	20	Eugenol	62
Linalool	22	Linalyl acetate	20	1,8-cineole	16	Acetyleugenol	18
Cis-ocimene	7	1,8-cineole	7	Camphor	15	Caryophyllene	17
Lavandulyl acetate	5	Camphor	6	α-thujone	9	Humulene	2
Farnesen	5	*trans*-β-ocimene	4	Camphene	7	α-copaene	1
C. sempervirens	**C%**	***A. sativum***	**C%**	***P. anisum***	**C%**		
α-pinen	44	Diallyl disulphide	77	*trans*-anethole	87		
β-pinen	11	Diallyl sulphide	16	Estragole	5		
Δ-3-carene	11	Methyl allyl disulfide	3	Pseudoisoueugenol-2-methyl butyrate	3		
Limonen	10	Methyl allyl trisulfide	2	Muurolene	1		
Bornyl acetate	7			Cis-anethole	1		

C%: Concentration (%)

Antibacterial activity

According to the antibacterial activity results, generally all essential oils exhibited the greatest inhibition zones for Gram-positive bacteria than for Gram-negative bacteria. In the current study, highest inhibition zones were determined by *P. anisum* (24 mm), *C. sempervirens* (36 mm) and *S. officinalis* (37 mm) essential oils against *S. aureus* ATCC 25923; *L. intermedia* (30 mm) and *S. aromaticum* (28 mm) essential oils against *E. coli* NRRLB 3704; *L. angustifolia* (65 mm) and *A. sativum* (20 mm) essential oils against *B. subtilis* ATCC 6633 (Table 2).

It was observed that *L. angustifolia* essential oil, exhibited antibacterial effect against 11 bacteria and *P. anisum* essential oil against only 3 bacteria. According to most literature, the essential oil activity is categorized as low activity at 10 mm, moderate activity at > 10 to 15 mm, and high activity at > 15 mm. The essential oils with inhibition zone diameter ≤ 8 mm can be considered non-efficient, whereas the essential oils with an inhibition zone ≥ 15 mm are considered very efficient, and ≥ 20 mm is considered extremely efficient (Abdelatti et al., 2023; El Hachlafi et al., 2023; Hulankova, 2024; Ponce et al., 2003; Rathore et al., 2022).

Therefore, it can be said that *L. angustifolia* essential oil has the most efficient antibacterial effect while essential oil of *P. anisum* has the lowest antibacterial effect. Additionally, essential oils extremely efficient activity against Gram-positive bacteria than Gram-negative bacteria.

Among the all tested bacteria, it was observed that *S. aureus* ATCC 25923 and *E. coli* NRRLB 3704 were the most sensitive strains against all essential oils. It was found that *A. hydrophilia* ATCC 95080 and *P. vulgaris* ATCC 13315 were sensitive against *L. angustifolia* with 20 mm and 45 mm inhibition zones respectively and they were sensitive against *S. aromaticum* with 10 and 20 mm inhibition zones. Also, it was observed that *A. hydrophilia* ATCC 95080 and *P. vulgaris* ATCC 13315 were resistant to other essential oils. According to the comparison of inhibition zones of essential oils and positive controls (streptomycin and penicillin), it was observed that most of the essential oils have more than antibacterial effects against most bacteria (Table 2).

Table 2. Inhibition zones (IZ) of different essential oils on bacteria

Inhibition zones (mm)									
Bacteria Species	***L. angustifolia***	***L. intermedia***	***C. sempervirens***	***S. aromaticum***	***S. officinalis***	***A. sativum***	***P. anisum***	**S**	**P**
Gram-Positive Bacteria									
S. aureus ATCC 25923	37	27	**36**	24	**37**	14	**24**	25	12
S. aureus ATCC 29213	55	10	30	15	15	-	-	-	10
S. haemolyticus ATCC 43252	20	22	22	15	-	-	-	11	10
E. faecalis ATCC 29212	11	20	20	-	13	18	-	20	-
B. subtilis ATCC 6633	**65**	15	20	14	11	**20**	-	19	22
Gram-Negative Bacteria									
P. aeruginosa ATCC 27853	15	18	15	20	-	-	-	7	20
K. pneumoniae ATCC 700603	22	10	7	13	-	-	-	17	-
E. coli NRRLB 3704	22	**30**	14	**28**	29	8	16	20	18
A. baumannii ATCC 19606	50	12	27	27	20	-	12	10	-
P. vulgaris ATCC 13315	45	-	-	20	-	-	-	10	15
A. hydrophila ATCC 95080	20	-	-	10	-	-	-	18	16

S: Streptomycin, **P:** Penicillin

The inhibition zone diameters of the combinations obtained from essential oils are given in Table 3. The highest inhibition zone diameters were determined to be against, *S. aureus* ATCC 25923 (70 mm) in combination A, *K. pneumoniae* ATCC 700603 (25 mm) in combination B, *S. haemolyticus* ATCC 43252 (45 mm) in combination C, *E. coli* NRRLB 3704 (40) mm) in combination D, *P. aeruginosa* ATCC 27853 (40 mm) in combination E, and *S. haemolyticus* ATCC 43252 (20 mm) in combination F.

Synergistic effects of essential oil blends and fold percentage increase results (%) were determined (Table 4). The increased fold percentage for *L. angustifolia* with combinations was as follow; for *L. intermedia* (combined A) against 89,18% *S. aureus* ATCC 25923, 65% *S. haemolyticus* ATCC 43252 and 66,6% *P. aeruginosa* ATCC 27853; for *C. sempervirens* (combined B) against 26,6% *P. aeruginosa* ATCC 27853 and 13.6% *K. pneumoniae* ATCC 700603; for *S. aromaticum* (combined C) against 125% *S. haemolyticus* ATCC 43252, 36,3% *K. pneumoniae* ATCC 700603, 27,7% *E. coli* NRRLB 3704, 10% *A. hydrophylia* ATCC 95080 and 9,09%. *E. faecalis* ATCC 29212; for *S. officinalis* (combined D) against 59,09% *K. pneumoniae* ATCC and 81,8% *E. coli* NRRLB 3704; for *P. anisum* (combined E) against 166,7% *P. aeruginosa* ATCC 27853 and 18,18%. *E. faecalis* ATCC 29212. The increased fold percentage for *L. angustifolia* with *A. sativum* (combined F) against all bacteria were negative.

Resulting combinations antibacterial effects were evaluated for antagonistic, synergistic or additive effects. As a result, it was determined that combinations A, C, and E had a synergistic effect on a total of 3 Gram-positive bacteria, and combinations A, B, C, D, and E had a synergistic effect on 4 Gram-negative bacteria. This indicates that while the essential oils were effective against Gram-positive bacteria individually, they were more effective against Gram-negative bacteria when used in combination. Combined F (with *A. sativum*) had antagonistic antibacterial effect on all tested bacteria. The highest percentage of fold increase was found for combined E on *P. aeruginosa* ATCC 27853 (166,7%). It was also observed that the *L. angustifolia-S. aromaticum* (combined C) mixture had synergistic effect on 5 bacteria (*K. pneumoniae* ATCC 700603 *E. coli* NRRLB 3704, *A. hydrophila* ATCC 95080, *E. faecalis* ATCC 29212 and *S. haemolyticus* ATCC 43252). However, no synergistic or additive effects were observed against *S. aureus* ATCC 29213, *B. subtilis* ATCC 6633, *A. baumannii* ATCC 19606 and *P. vulgaris* ATCC 13315 strain in all tested combinations. According to these combination results we can say that synergistic effects were especially on the bacterial strains that cause hospital infection.

Table 3. Inhibition zones (IZ) of *L. angustifolia* and other essential oil combines on bacteria

	Inhibition zones (mm)					
	A	**B**	**C**	**D**	**E**	**F**
S. aureus ATCC 25923	**70**	15	30	17	10	18
S. aureus ATCC 29213	11	10	20	6	10	-
S. haemolyticus ATCC 43252	33	19	**45**	18	13	**20**
E. faecalis ATCC 29212	11	9	12	10	13	-
B. subtilis ATCC 6633	12	17	20	12	-	17
P. aeruginosa ATCC 27853	25	19	15	15	**40**	14
K. pneumoniae ATCC 700603	12	**25**	30	35	-	-
E. coli NRRLB 3704	17	18	28	**40**	12	-
A. baumannii ATCC 19606	39	15	16	12	-	19
P. vulgaris ATCC 13315	10	14	15	7	-	-
A. hydrophila ATCC 95080	12	8	22	-	-	14

A: *L. angustifolia* and *L. intermedia*, **B:** *L. angustifolia* and *C. sempervirens*, **C:** *L. angustifolia* and *S. aromaticum*, **D:** *L. angustifolia* and *S. officinalis*, **E:** *L. angustifolia* and *P. anisum*, **F:** *L. angustifolia* and *A. sativum*

Table 4. Synergistic effects of essential oil blends and fold percentage increase (%)

Microorganisms	A Combination Effect	Fold increase %	B Combination Effect	Fold increase %	C Combination Effect	Fold increase %	D Combination Effect	Fold increase %	E Combination Effect	Fold increase %	F Combination Effect	Fold increase %
S. aureus ATCC 25923	PSAE	89,18	Antagonism	-59,46	Antagonism	-18,92	Antagonism	-54,05	Antagonism	-0,73	Antagonism	-0,52
S. aureus ATCC 29213	Antagonism	-80	Antagonism	-81,8	Antagonism	-63,6	Antagonism	-89,1	Antagonism	-81,8	Antagonism	-100
S. haemolyticus ATCC 43252	PSAE	65	Antagonism	-5	PSAE	125	Antagonism	-10	Antagonism	-35	Antagonism	0
E. faecalis ATCC 29212	Antagonism	0	Antagonism	-18,18	PSAE	9,09	Antagonism	-9,09	PSAE	18,18	Antagonism	-100
B. subtilis ATCC 6633	Antagonism	-81,5	Antagonism	-73,8	Antagonism	-69,2	Antagonism	-81,5	Antagonism	-100	Antagonism	-73,8
P. aeruginosa ATCC 27853	PSAE	66,6	PSAE	26,6	Antagonism	0	Antagonism	0	PSAE	166.7	Antagonism	-6,66
K. pneumoniae ATCC 700603	Antagonism	-45,5	PSAE	13,6	PSAE	36,3	PSAE	59,09	Antagonism	-100	Antagonism	-100
E. coli NRRLB 3704	Antagonism	-22,7	Antagonism	-18,2	PSAE	27,27	PSAE	81,8	Antagonism	-45,45	Antagonism	-100
A. baumannii ATCC 19606	Antagonism	-22	Antagonism	-70	Antagonism	-68	Antagonism	-76	Antagonism	-100	Antagonism	-62
P. vulgaris ATCC 13315	Antagonism	-77,7	Antagonism	-68,8	Antagonism	-66,6	Antagonism	-84,4	Antagonism	-100	Antagonism	-100
A. hydrophila ATCC 95080	Antagonism	-40	Antagonism	-60	PSAE	10	Antagonism	-100	Antagonism	-100	Antagonism	-30

A: *L. angustifolia* and *L. intermedia*, **B:** *L. angustifolia* and *C. sempervirens*, **C:** *L. angustifolia* and *S. aromaticum*, **D:** *L. angustifolia* and *S. officinalis*, **E:** *L. angustifolia and P. anisum*, **F:** *L. angustifolia* and *A.sativum*;
Antagonism: A substance has an antagonistic effect; **PSAE:** Potential Synergistic or Additive Effect

In the literature, there are a lot of different herbal essential oil studies including their antioxidant and antimicrobial activities (El-Mesallamy et al., 2012; Romoeo et al., 2008; Tardugno et al., 2018). Antimicrobial properties of various essential oils like lavender (*Lavandula angustifolia* Mill.), lemon-balm (*Melissa officinalis* L.), juniper berry (*Juniperus* communis L.), lemon verbena (*Lippia citriodora* Kunth), rosemary (*Rosmarinus officinalis* L.) and cypress (*Cupressus sempervirens* L.) were investigated. Essential oils' antimicrobial activity depends on the source plant, composition and concentration of the oils, the type and concentration of the target microorganism (Burt et al., 2025; Pandit et al., 1994; Skandamis et al., 2002). *L. angustifolia* is known to have a broad antibacterial effect against Gram-positive and Gram-negative bacteria (Blašković et al., 2018; Raut et al., 2025). Blazkekovic et al. in 2018 determined the antibacterial effect of *L. angustifolia* and *L. intermedia* essential oils (EO) by agar well diffusion method and they reported that *L. intermedia* essential oil exhibited antibacterial effect against *S. aureus*, *E. coli*, *K. pneumonia*, *P. aeruginosa* and *E. faecalis* strains (Blašković et al., 2018). This study supports our results but we found greater inhibition zones for *S. aureus*, *K. pneumonia* and *E. coli* strains. It can be associated with composition differences between tested essential oils. Blašković et al. studied with the essential oils with high linalool concentrations. Our essential oil exhibited nearly half the linalool concentration compared to the study by Blašković et al. However, our essential oils' linalyl concentrations were higher than Blazekovic's. In comparison to other compounds investigated, the fundamental compositional differences between the oils tested in the two studies were primarily observed as a high camphor concentration in the *L. intermedia* sample and a high cis-ocimene content in the *L. angustifolia* sample of the oils utilized in our study. Lis-Balchin et al. investigated the antibacterial effect of *P. anisum* and *S. aromaticum* EO by agar well diffusion, they reported that *P. anisum* EO exhibited low antibacterial effect against *A. hydrophila* with no antibacterial effect against *B. subtilis*, *E. coli* and *S. aureus* strains. Also, they observed antibacterial effect of *S. aromaticum* EO against *A. hydrophila*, *B. subtilis*, *E. coli* and *S. aureus* strains (Lis-Balchin et al., 1998). On the contrary, in our study *P. anisum* EO exhibited 16 mm and 24 mm inhibition zones for *E. coli* and *S. aureus* strains. *S. aromaticum* EO exhibited greater antibacterial effect against *E. coli* and *S. aureus* strains in our antibacterial activity test. Khubeiz et al. studied the antibacterial effect of *C. sempervirens* leaves essential oil (Khubeiz et al., 2016). They determined 42,30 mm, 10,20 mm, 9,40 mm inhibition zones against *S. aureus*, *K. pneumonia*, and *P. aeruginosa* respectively. They didn't observe an inhibition zone against *B. subtilis*, *P. vulgaris*, and *E. coli*. Unlike, according to our results, *C. sempervirens* essential oil exhibited an antibacterial effect against *B. subtilis* and *E. coli*. Thought that the difference between the results of the two studies may be due to factors such as the different bacterial strains tested or the different content, method of obtaining and purity of the *C. sempervirens* essential oil used in the tests. They found α-pinen as the major compound (36.50%), followed by 3-carene (22.17%) and germacrene D (12.81%). In our essential oil, α-pinene was also the major compound, however, the difference was mainly on germacrene and pinene concentrations. Our essential oil has less germacrene concentration and higher β-pinene concentrations. In another study, Fu et al. determined antibacterial activity of *S. aromaticum* and *S. rosmarinus* EO by agar disc diffusion method (Fu et al., 2007). According to results, *S. epidermidis*, *S. aureus*, *B. subtilis*, *E. coli* and *P. vulgaris* strains were found sensitive against *S. aromaticum* EO. Contrary, in our study inhibition zone for *S. epidermidis* was not observed. *S. aureus*, *E. coli* and *P. aeruginosa* were found more sensitive against *S. aromaticum* in our study. Khedher et al. investigated the chemical and biological activity of *S. officinalis* EO and they reported 14 mm, 12 mm, 14 mm, 12 mm inhibition zones against *B. cereus*, *B. subtilis*, *S. aureus*, and *E. coli* (Khedher et al., 2017). In our study, stronger inhibition zones for *S. aureus* and *E. coli* were measured. The major compounds of the essential oil tested were camphor (25.14%) and α-thujone (18.83%), followed by 1,8-cineole (14.14%) and to lesser concentration viridiflorol (7.98%). Only 1,8-cineole concentration was somewhat similar to our essential oil concentration. Chekki et al. investigated the antibacterial properties of *A. sativum* essential oil and extracts and they didn't observe inhibition zone for *E. coli* and *P. aeruginosa* (Chekki et al., 2014). However, they obtained 12 mm inhibition zone for *S. aureus*. In our study, we observed 14 mm and 8 mm, inhibition zones for *S. aureus*, and *E. coli* strains while no inhibition zone was observed for *P. aeruginosa*.

The primary distinction of our study from the aforementioned antibacterial activity research using essential oils is the investigation of the combined effects of these oils against important pathogenic bacteria. This approach significantly illuminates the synergistic potential of essential oils against pathogens.

Conclusion

The study results indicate that the chemical composition of each essential oil is unique, and therefore, their antimicrobial effects vary. Furthermore, blending different essential oils in different proportions and types can increase or, conversely, decrease the antimicrobial activity of the essential oil. Essential oil combination can create powerful synergistic effects in terms of antibacterial properties and may emerge as a natural alternative, particularly in the fight against antibiotic resistance. Therefore, it is recommended that each essential oil be tested against microorganisms, both individually and in combination with other essential oils, before being used in aromatherapy. This will allow essential oils to be used as an adjunct or alternative to antibiotics in combating many infectious microorganisms.

Author contributions

DD: Supervision; İD: LC-MS analyses; DD, ŞMÖA: Experimental analysis; İD: Essential oil extraction; DD, VS, ŞMÖA, EM: Writing, data analyses, editing.

Declaration of interests

The authors declare no conflict of interest.

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