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Research Article

KIRŞEHİR EKOLOJİK KOŞULLARINDA YETİŞTİRİLEN *LAVANDULA ANGUSTİFOLİA* L. VE *SALVİA OFFİCİNALİS* L. BİTKİLERİNİN UÇUCU YAĞLARININ KLİNİK ÖNEME SAHİP PATOJENLERE KARŞI ANTİBAKTERİYEL POTANSİYELLERİNİN DEĞERLENDİRİLMESİ

EVALUATİON OF THE ANTİBACTERİAL POTENTİALS OF ESSENTİAL OİLS FROM *LAVANDULA ANGUSTİFOLİA* L. AND *SALVİA OFFİCİNALİS* L. CULTİVATED UNDER THE ECOLOGİCAL CONDİTİONS OF KIRŞEHIR AGAİNST CLİNİCALLY SİGNİFİCANT PATHOGENS

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Makale Bilgileri	**Özet**
Anahtar Kelimeler: *Lavandula angustifolia* ve *Salvia officinalis.* Uçucu yağ, Antimikrobiyal aktivite	Geleneksel antimikrobiyal ilaçların olası yan etkileri ve bakteriyel direnç gelişimi, halk sağlığı açısından ciddi bir tehdit oluşturmaktadır. Bu durum, doğal ve bitkisel kaynaklı alternatiflerin araştırılmasını giderek daha önemli hâle getirmiştir. Günümüzde birçok aromatik ve tıbbi bitkinin, yalnızca beslenme amacıyla değil, aynı zamanda enfeksiyonlara karşı koruyucu olarak halk tarafından yaygın biçimde kullanıldığı bilinmektedir Mevcut çalışmada, Kırşehir koşullarında kültüre alınan *Lavandula angustifolia* (lavanta) ve *Salvia officinalis* (adaçayı) bitkilerinden elde edilen uçucu yağların antimikrobiyal etkileri değerlendirilmiştir. Uçucu yağların antibakteriyel aktiviteleri, yedi farklı klinik öneme sahip bakteri suşuna karşı disk difüzyon yöntemi, MİK (Minimum İnhibitör Konsantrasyonu) ve MBK (Minimum Bakterisidal Konsantrasyon) analizleri ile incelenmiştir. Elde edilen sonuçlar, her iki bitkinin de hem Gram-pozitif hem de Gram-negatif bakterilere karşı anlamlı düzeyde antimikrobiyal aktivite gösterdiğini ortaya koymuştur. *L. angustifolia* uçucu yağı, özellikle *B. cereus* (25 mm) ve *S. aureus* (20 mm) üzerinde geniş inhibisyon zonları oluşturarak daha yüksek etki göstermiştir. MIC değeri *E. coli ve E. faecalis* için %0.39 olarak belirlenmiş, bu da düşük konsantrasyonda dahi etkili olduğunu ortaya koymuştur. *S. officinalis* uçucu yağı ise en düşük MIC değerini *S. aureus* suşunda (%0.39) göstermiştir. Sonuçlar, Kırşehir ikliminde yetiştirilen bu bitkilerden elde edilen uçucu yağların, dirençli

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bakterilerle mücadelede potansiyel doğal antimikrobiyal ajanlar olarak değerlendirilebileceğini göstermektedir.

Article Info	Abstract
Keywords:*Lavandula angustifolia* and *Salvia officinalis*, essential oil, antibacterial activity.	The potential side effects of traditional antimicrobial drugs and the development of bacterial resistance pose a serious threat to public health. This situation has made the search for natural and plant-based alternatives increasingly important. Today, many aromatic and medicinal plants are widely used by the public not only for nutritional purposes but also as protective agents against infections. In the present study, the antimicrobial effects of essential oils obtained from *Lavandula angustifolia* (lavender) and *Salvia officinalis* (sage), cultivated under the ecological conditions of Kırşehir, were evaluated. The antibacterial activities of the essential oils were investigated against seven clinically significant bacterial strains using the disk diffusion method, MIC (Minimum Inhibitory Concentration), and MBC (Minimum Bactericidal Concentration) analyses. The results revealed that both plants exhibited significant antimicrobial activity against both Gram-positive and Gram-negative bacteria. *L. angustifolia* oil stood out with broader inhibition zones and lower MIC values, particularly against *L. angustifolia* essential oil showed higher efficacy, particularly forming large inhibition zones against *B. cereus* (25 mm) and *S. aureus* (20 mm). The MIC value was determined to be 0.39% for *E. coli* and *E. faecalis*, indicating effectiveness even at low concentrations. *S. officinalis* essential oil exhibited its lowest MIC value (0.39%) against the *S. aureus* strain. The findings suggest that the essential oils obtained from these plants cultivated in the Kırşehir climate could be considered as potential natural antimicrobial agents in the fight against resistant bacteria.

1. INTRODUCTION

The increasing prevalence of infections caused by multidrug-resistant (MDR) bacteria has emerged as a serious global public health concern, particularly as existing treatment options become increasingly limited. The widespread and often inappropriate use of antibiotics has accelerated the emergence and dissemination of resistant strains, thereby contributing to a global rise in morbidity and mortality rates (Mohammadinejad et al., 2019). This alarming situation has heightened interest in novel therapeutic approaches, especially those derived from natural products with antimicrobial potential.

In today's world, where the search for alternative treatments is on the rise, antimicrobial resistance observed in both Gram-positive and Gram-negative bacteria continues to challenge modern medicine. In particular, species such as methicillin-resistant *Staphylococcus aureus* (MRSA) and *Bacillus cereus* play a significant role in healthcare-associated infections such as pneumonia, wound infections, and bacteremia, complicating the treatment process and increasing the burden on healthcare systems (Mahjabeen et al., 2022). Similarly, multidrug-resistant (MDR) Gram-negative pathogens such as *Escherichia coli* and *Klebsiella pneumoniae* that produce extended-spectrum β-lactamases (ESBLs) are resistant to key antibiotic groups such as β-lactams, fluoroquinolones, and carbapenems, and represent significant clinical threats (Jean et al., 2022). Global surveillance studies have identified pathogens such as *Klebsiella pneumoniae, Escherichia coli, Enterococcus faecalis, and Staphylococcus aureus* as the leading causes of hospital-acquired infections (Jean et al., 2022; Santajit & Indrawattana, 2016).

In light of these challenges, natural products, and especially essential oils, are emerging as promising alternatives in the management of multidrug-resistant infections. Thanks to their antimicrobial properties attributed to their rich and diverse chemical structures, they have the potential to play an important role in the development of new therapeutic strategies (Mohammadinejad et al., 2019). Essential oils are secondary metabolites with a certain phytochemical composition obtained from different parts of various aromatic plants (Alizadeh Behbahani & Shahidi, 2019; İşcan et al., 2002; Katar et al., 2020; Lal et al., 2020). Plants that have adapted to different ecological conditions generally exhibit remarkable phytochemical diversity. This chemical richness, shaped by both environmental and genetic factors, has made aromatic plants important sources of bioactive compounds (Arzani & Ashraf, 2016; Soleimani et al., 2022). In this context, the species *Lavandula angustifolia* (lavender) and *Salvia officinalis* (sage) belonging to the Lamiaceae family are noteworthy due to their traditional medicinal uses and the therapeutic effects of their essential oils. The essential oils of these widely cultivated perennial plants have been used for centuries in the treatment of conditions such as ulcers, spasms, inflammation, dizziness, and rheumatism (Abou Baker et al., 2021; Benbrahim et al., 2021; Ezema et al., 2022).

The essential oils of *Lavandula angustifolia* and *Salvia officinalis* species generally contain main components such as 1,8-cineole and borneol; they can exhibit significant chemotypic differences depending on the species, geographical origin, harvest time, and cultivation conditions (Abou Baker et al., 2021; Andrys et al., 2018; Arnao et al., 2001). These chemical differences can directly affect the antimicrobial efficacy of essential oils.

In this study, the in vitro antibacterial activities of essential oils obtained from *Lavandula angustifolia* and *Salvia officinalis* plants against various multidrug-resistant bacterial strains will be evaluated. These two plant species have been cultivated for the first time under the ecological conditions of Kırşehir Province and included in this study. This situation provides a unique contribution to the literature in terms of revealing the antimicrobial potential of these plants based on the secondary metabolite profiles they synthesize under the climatic conditions of Kırşehir.

2. MATERIALS and METHODS

Plant Material

The *Lavandula angustifolia* and *Salvia officinalis* plants used in this study were cultivated at the AHİGETAM Medical and Aromatic Plants Application and Research Area of Kırşehir Ahi Evran University. *L. angustifolia* was harvested at full bloom, while *S. officinalis* was harvested at the beginning of flowering. The harvested plants were dried in a shaded environment at room temperature under conditions that ensured air circulation. The dried samples *were ground in a grinder to achieve homogeneity.*

Extraction of Essential Oils

The hydrodistillation method was used to extract essential oils from *L. angustifolia* and *S. officinalis* plants. A Clevenger-type apparatus was used for this process. The distillation process was carried out for approximately 3 hours. The condensed phases were separated due to differences in specific gravity, and the essential oils were collected. The obtained essential oils were passed through anhydrous sodium sulfate and then stored in amber-colored glass bottles at 4°C until analysis was performed.

Test Microorganisms

The microorganisms evaluated for antibacterial activity in this study consisted of reference strains obtained from the Medical Biology Laboratory of the Faculty of Medicine at Kırşehir Ahi Evran University. The bacterial strains used were as follows: *Klebsiella pneumoniae* (ATCC 10031), *Escherichia coli* (ATCC 25922), *Yersinia pseudotuberculosis* (ATCC 911), *Bacillus subtilis* subsp. *spizizenii* (ATCC 6633), *Staphylococcus aureus* (ATCC 25213), beta-toxin-positive *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), and *Bacillus cereus* (709 Roma isolate). All strains were cultured in appropriate media prior to the experiment to activate them, and pure cultures were used throughout the study.

Antibacterial Activity Test

The antibacterial effects of essential oils were evaluated using the disk diffusion method. The Kirby-Bauer disk diffusion method, used in the preliminary evaluation of antibacterial activity, is a widely used and standardized technique for determining the susceptibility of microorganisms to various antibiotics. In this method, antibacterial activity is evaluated by measuring the diameter of the inhibition zones, and the results are interpreted according to standards such as CLSI (Yao et al., 2021). Each bacterial strain was prepared to a 0.5 McFarland standard (approximately 1×10^8 CFU/mL) and evenly spread on the surface of Mueller-Hinton agar (MHA; Merck, Germany) plates using a sterile cotton swab. Each sterile 6 mm diameter disc was impregnated with 10 µL of essential oil prepared at a concentration of 25% (v/v), and these discs were placed on the surface of the inoculated bacterial layer. Ampicillin at a concentration of 1 mg/mL was used as a positive control, and a 5% DMSO solution was used as a negative control. To evaluate the antibacterial activity against bacterial growth, the diameters of the inhibition zones formed around the wells were measured using a standard antibiogram chart after 24 hours of incubation at 37°C.

Minimum Inhibitory Concentration (MIC) Determination

Minimum inhibitory concentrations (MIC) were determined using the broth microdilution method according to CLSI guidelines (Weinstein, 2018). Essential oils were serially diluted (12.5–0.098%) in Mueller-Hinton broth (MHB) containing 5% (v/v) DMSO, and 10^6 CFU/mL bacterial suspension was added to each well. The bacterial strains were incubated at 37°C for 24 hours, and growth observations were evaluated visually.

Determination of Minimum Bactericidal Concentration (MBC)

In order to determine the minimum bactericidal concentration (MBC) values, concentrations equal to or higher than the previously determined MIC value were selected. To evaluate bactericidal activity against these concentrations, inoculum was taken from the relevant microdilution wells using a sterile loop and inoculated into Petri dishes containing fresh medium (Mueller-Hinton agar). After incubation at 37°C for 24 hours, the lowest concentration at which no colony growth was observed was accepted as the MBC.

3. RESULTS

The antibacterial activity of essential oils obtained from *L. angustifolia* and *S. officinalis* plants was evaluated against pathogenic Gram-positive and Gram-negative bacterial strains. In this context, inhibition zone diameters and MIC and MBC values are presented in Table 1 and Table 2, respectively. It was determined that DMSO, used as a solvent in the experiments, did not exhibit any antimicrobial effect on the tested microorganisms. The essential oils demonstrated moderate to high levels of antibacterial activity, forming inhibition zones ranging from 13 to 25 mm against the seven different pathogenic bacterial strains tested. However, *L. angustifolia* essential oil exhibited a stronger antibacterial effect profile than Salvia officinalis essential oil, forming wider inhibition zones against most of the tested bacterial strains.

Among Gram-negative bacteria, *L. angustifolia* essential oil produced inhibition zones of 15 mm and 14 mm against *E. coli* and *K. pneumoniae* strains, respectively, while *S. officinalis* essential oil produced zone diameters of 14 mm and 13 mm against the same strains, respectively. Against *Y. pseudotuberculosis* strains, *L. angustifolia* essential oil produced a zone of 16 mm, which is higher than the 14 mm zone diameter produced by *S. officinalis* essential oil. This result indicates that *L. angustifolia* essential oil is more effective than *S. officinalis* essential oil against all tested Gram-negative bacteria.

When evaluated in terms of Gram-positive bacteria, *L. angustifolia* essential oil showed the highest antibacterial effect against *B. cereus* strain with a zone diameter of 25 mm. *S. officinalis* essential oil produced a zone diameter of 16 mm against the same strain. Additionally, *L. angustifolia* essential oil formed a 20 mm zone against the *S. aureus* strain, showing a similar effect to ampicillin (19 mm). In the *S. aureus* BT strain, both essential oils produced inhibition zones of equal size (15 mm). Against the *E. faecalis* strain, *L. angustifolia* and *S. officinalis* essential oils exhibited similar levels of antimicrobial activity, producing zones of 17 mm and 16 mm, respectively. These findings support that *L. angustifolia* essential oil generally exhibits stronger antimicrobial activity against both Gram-negative and Gram-positive bacteria compared to *S. officinalis* essential oil.

Table 1. Inhibition zone diameters of *Lavandula angustifolia* ve *Salvia officinalis* essential oils against tested bacterial strains

Microorganisms	Zone Diameters (mm)		
	***L.angustifolia* EO**	***S. officinalis* EO**	**Ampicillin**
Gram-negative bacteria			
E. coli (ATCC 25922)	15	14	20
K. pneumoniae (ATCC 10031)	14	13	22
Y. pseudotuberculosis (ATCC 911)	16	14	21
Gram-positive bacteria			
S. aureus (ATCC 25213)	20	15	19
S. aureus BT *(ATCC 25923)*	15	15	21
E. faecalis (ATCC 29212)	17	16	22
B. cereus (709 Roma)	25	16	21

EO: Essential oil

To support the findings obtained by the disk diffusion method, the antimicrobial activities of L. angustifolia and S. officinalis essential oils were quantitatively evaluated based on MIC and MBC values (Table 2). This study reveals that both essential oils possess potential antibacterial properties against bacterial pathogens. L. angustifolia essential oil exhibited the lowest inhibitory concentration with an MIC value of 0.39% against E. coli and E. faecalis strains. S. officinalis essential oil exhibited the lowest MIC value of 0.39% against the S. aureus strain. These values indicate that both essential oils possess high antibacterial potential against the aforementioned strains.

While *L. angustifolia* essential oil showed efficacy against *B. cereus* strain with MIC and MBC values of 0.78% and 3.125%, respectively, the bactericidal effect of *S. officinalis* essential oil against this strain appeared at a relatively higher concentration of 6.25%. However, this value still indicates strong activity when considering the natural structure of essential oils.

Table 2. MIC and MBC values of *Lavandula angustifolia* ve *Salvia officinalis* essential oils against tested bacterial strains

Microorganisms	MIC Values (%)		MBK Values (%)	
	***L.angustifolia* EO**	***S. officinalis* EO**	***L.angustifolia* EO**	***S. officinalis* EO**
E. coli (ATCC 25922)	0.39	0.78	0.78	1.56
K. pneumoniae (ATCC 10031)	1.56	1.56	1.56	3.125
Y. pseudotuberculosis (ATCC 911)	1.56	1.56	1.56	3.125
S. aureus (ATCC 25213)	0.78	0.39	3.125	1.56
S. aureus BT *(ATCC 25923)*	0.78	0.78	3.125	3.125
E. faecalis (ATCC 29212)	0.39	0.78	3.125	3.125
B. cereus (709 Roma)	0.78	1.56	3.125	6.25

EO: Essential oil

4. DISCUSSION

In this study, the antimicrobial effects of essential oils obtained from *Lavandula angustifolia* and *Salvia officinalis* plants were evaluated against both Gram-positive and Gram-negative bacteria, and the results revealed that these natural compounds have significant antibacterial potential.

The bacteria tested are clinically important infectious agents in terms of human health. *E. coli* and *K. pneumoniae* are Gram-negative strains that cause serious infections such as urinary tract infections, sepsis, and hospital-acquired pneumonia (Diekema et al., 2019). *S. aureus* and *B. cereus* cause a wide range of diseases, from skin and soft tissue infections to foodborne illnesses (Khan et al., 2015); *E. faecalis* is a Gram-positive bacterium primarily responsible for serious diseases such as catheter-related infections and endocarditis (Xie et al., 2024). *Y. pseudotuberculosis* is a globally prevalent Gram-negative pathogen that can cause gastrointestinal infections such as gastroenteritis and mesenteric lymphadenitis in humans (Fredriksson-Ahomaa, 2009).

Disk diffusion analyses have shown that *L. angustifolia* essential oil exhibits broad-spectrum antimicrobial activity, particularly against clinically critical strains such as *B. cereus* (25 mm), *S. aureus* (20 mm), and *E. coli* (15 mm). *S. officinalis* essential oil produced narrower zone diameters but still exhibited significant antimicrobial activity. The primary reason for the efficacy of essential oils lies in their biologically active components. Chromatographic analysis findings in the literature reveal that the main components of *L. angustifolia* essential oil are linalool and linalyl acetate, while *S. officinalis* essential oil contains 1,8-cineole, camphor, and cis-thujone (Mourabiti et al., 2024). It has been reported that linalool disrupts cell membrane integrity, causing intracellular contents to leak out, thereby inhibiting the vital functions of bacteria (Eliuz et al., 2016). Similarly, it is known that 1,8-cineole and camphor disrupt protein synthesis and enzyme activity (Jirovetz et al., 2005).

In MIC/MBC analyses, the fact that L. angustifolia essential oil is effective against *E. coli* and *E. faecalis* strains at low concentrations of 0.39% indicates that it has a strong effect profile against both Gram-negative and Gram-positive bacteria. *S. officinalis* essential oil, on the other hand, exhibited the lowest MIC value against *S. aureus* strains (0.39%), suggesting that it may be more effective against Gram-positive pathogens in particular.

When these data are considered together, it is understood that the antibacterial potential of essential oils depends on both their content and the structural characteristics of the target bacterial species. The higher effect of *L. angustifolia* essential oil is probably due to the synergistic destruction effect of linalool and linalyl acetate on the cell membrane. The components of *S. officinalis* essential oil, on the other hand, appear to be particularly effective against Gram-positive bacteria. The *L. angustifolia* and *S. officinalis* plants used in the study were cultivated in the Kırşehir region, and their essential oils were obtained from these samples. In this regard, the study is significant in terms of evaluating the antimicrobial potential of essential oils obtained from locally sourced plant material. In this respect, the study provides an important contribution in terms of revealing the antimicrobial potential of essential oils obtained from *L. angustifolia* and *S. officinalis* plants cultivated in the ecological conditions of Kırşehir. The findings show that the phytochemical profiles of these plants developing in the Kırşehir climate may play a decisive role in their antibacterial activities and provide a scientific basis for the potential applications of these essential oils in the field of health.

5. CONCLUSIONS

This study has demonstrated the antimicrobial effects of essential oils obtained from *Lavandula angustifolia* and *Salvia officinalis* plants cultivated in Kırşehir ecological conditions against both Gram-positive and Gram-negative bacteria. As a result of disk diffusion, MIC and MBC analyses, it was determined that especially *L. angustifolia* essential oil has a broad-spectrum and strong antibacterial effect. This effect is thought to be due to phytochemical components such as linalool and linalyl acetate contained in the essential oil. *S. officinalis* essential oil also showed a significant effect against some Gram-positive bacteria, especially *Staphylococcus aureus*. The fact that both essential oils were effective at low concentrations suggests that these natural compounds can be evaluated as alternative antimicrobial agents in the fight against multidrug-resistant pathogens. In conclusion, this study; It contributes to the literature by showing the strong antibacterial potential of the essential oils obtained from *L. angustifolia* and *S. officinalis* plants cultivated in Kırşehir province and also provides a scientific basis for advanced research on the use of these oils in the field of health.

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Conflict of Interest: There is no conflict of interest among the authors.

Data Availability: The data obtained in this study are presented in tables within the article and are clearly provided to support all analyses.

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