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Bioautography for evaluation of several *Lavandula* L. and *Origanum* species antimicrobial and antioxidant activity

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ABSTRACT

In the search of bioactive natural compounds, bioautography of plant extracts were associated in an antioxidant screening. Due to containing variety of phenolic compounds *Lavandula* and *Origanum* species are important medicinal plants. The antioxidant and free radical scavenging activities of *Lavandula angustifolia, L. stoechas, L. heterophylla, Origanum majorana, O. onites, O. vulgare, O. minituflorum,* and their main phenolic compounds linalool and carvacrol was carried out by TLC-bioautography method based on the $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assays to compare essential oils and known main active constituents. The antimicrobial activity of the materials was tested using the in vitro broth microdilution assay towards two different microorganisms. Methicillin-resistant *Staphylococcus aureus* and *Streptococcus mutans* were used for the study. As a result of our studies, it is determined that *O. vulgare* showed the highest activity against *S. mutans* and *O. onites* and *O. vulgare* showed the highest activity against MRSA. compared to the tested antibiotic.

Keywords: *Lavandula, Origanum*, essential oil, bioautography, antioxidant

INTRODUCTION

In Türkiye the extensive use of aromatic and medicinal plant species as primary remedies of the local culture, covering a considerable area with different

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environmental conditions, is quite common [1]. The Lamiaceae family, incorporates rich source of plants containing wide variety of phenolic acids and terpenes which has medical applications in the Mediterranean region [2,3]. *Lavandula L.* species are widely used in folk medicine and industrial fields[4]. Over the last decades, phenolic compounds and essential oils (EO) from these species is expanding for cosmetic and pharmaceutical uses [5,6]. Recent phytochemical investigations on *Lavandula* species oils revealed that monoterpenes are major components of the fractions with high contents of linalool, linalyl acetate 1,8-cineole, camphor, carvacrol and fenchone [7,8]. Lately, due their economic values many researchers were investigated the phytochemical and pharmacological aspects of Lavandula species. Previous studies shown that the essential oils or extracts of lavender display a broad spectrum of bioactivities such as anti-bacterial, anti-fungal, antioxidant, anti-inflammatory, insecticide, sedative, and anti-cancer activies [8,9]. Additionally, based on latest literature, linalool has anxiolytic, anti-cholesterol, and antibacterial activity [10]. Previously, it is determined that *L. angustifolia,* and *L. stoechas* have significant antioxidant activity, however, lavender species generally possess low antioxidant activities relative to *Origanum* species [8]. The *Origanum L.* (Lamiaceae) genus include 38 species distributed around the Mediterranean region, although most of them are confined to the eastern Mediterranean area. *Origanum* species are defined by a wide variety of terpenic molecules and by the presence of chemical differences in essential oil composition [11,12]. *Origanum* sp. EO includes carvacrol, thymol, and γ-terpinene as major constituents [11,12]. Besides the ethnobotanical usages there are many studies shown various biological effects such as antimicrobial, cytotoxic, antifungal, insecticidal, antioxidant, anti-spasmodic, antitumoral, and analgesic activities of *Origanum* species were reported [13,14]. *Origanum* essential oils were found to be amongst the most effective antioxidant natural agents [15]. Antioxidant compounds play a crucial role in essential oils biological activities, which is justified by the involvement of oxidative stress in pathology. These attributes are because of the inherent ability of particularly phenols, and specifically carvacrol and thymol, to inhibit the aerobic oxidation of organic matter [15,16]. The use of bioautography combined with thin-layer chromatography (TLC) is a allows the detection of active components screening for the investigation of the antioxidant effect. This study aims to obtain the antioxidant activity of *Lavandula angustifolia*, *L. stoechas*, *L. heterophylla* (synonym *L. hybrida)*, *Origanum majorana, O.onites, O. vulgare,* and *O. minituflorum* essential oils with TLC bioautography method based on the $DPPH^{\cdot}$ and $ABTS^{\cdot+}$ assays, and to evaluate their antimicrobial activities via *in vitro* broth microdilution assay towards two different microorganisms.

METHODOLOGY

For the experiment, analytically approved commercial essential oils were kindly provided by a Turkish company, Doalinn. The GC–MS analyses were performed in our previously studies, analyzed by GC-FID and GC/MS Analysis of the Agilent 6890N GC and Agilent 5975 GC-MSD systems [17,18].

TLC–Fingerprinting and DPPH Bioautography

Chromatographic separation was carried out on silica gel 60 F254 chromatographic plates (20 cm × 10 cm) using 7:3 Hxn: EtOAc to develop TLC plates. The essential oils were dissolved in ethanol and spotted on the chromatographic plates, then developed using different mixtures for *Origanum* and *Lavandula* EOs. Plates were prepared as duplicates and one of the chromatographic plates was derivatized with Anisaldehyde reagent and then heated at 105°C, one was dipped into DPPH (0.2%), and one of the plates was dipped into ABTS reagent [19].

Antioxidant activities of the essential oils were evaluated with the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) and 2,2-Azino-bis(3-ethylbenzthiazoline-6-sulfonic) acid (ABTS) bioautography methods. The plates examined in daylight after 30 min. $DPPH^{\cdot}$ solutions were displayed in the form of yellow fluorescent bands with purple background, and ABTS solutions were displayed in the form of colorless or pink spots with a green background, which were easy to be identified and were of high sensitivity Additionally, the intensity of the colors can be measured with a chromameter [20,21].

The antibacterial potential was determined using the *in vitro* broth microdilution assay against methicillin-resistant *Staphylococcus aureus* and *Streptococcus mutans*. According to our knowledge TLC-fingerprinting of different *Origanum* species was studied before [19]. However, this is the first time that it researches four different *Origanum* species from Turkish flora and compares the activity-chemotype relationship between them.

Antimicrobial Activity

The *in vitro* antimicrobial activity was determined using the broth microdilution assay following the methods according to the Clinical and Laboratory Standards Institute to determine the minimum inhibitory concentrations (MIC) [22]. Methicillin-resistant *Staphylococcus aureus* (Clinical isolate) and *Streptococcus mutans* (ATCC 25175) strains were grown in Mueller Hinton Broth (MHB, Merck, Germany) in aerobic conditions at 37 °C for 24 h. Microorganisms were adjusted to 1×10^8 CFU/mL using McFarland No: 0.5 in sterile saline (0.85%)

solution. Stock solutions and serial dilutions of the test samples were prepared in dimethyl sulfoxide (DMSO). Final DMSO concentration was 1% in each well. The minimum non-reproductive concentration was reported as minimum inhibitory concentration (MIC, as μg/mL). Essential oils were studied with serial dilution starting from 1 mg/mL concentration and MIC values were calculated. 1 mg/mL used as the initial concentration. Amoxicillin serial dilution starting from 1 μg/mL and tetracycline serial dilution starting from 0.1 μg/mL concentration and MIC values were calculated. The MIC was calculated and reported as the mean of three repetitions compared to positive standards as shown in Table 1 and Table 2.

RESULTS AND DISCUSSION

GC/MS and GC-FID analyses

The essential oil compositions of the tested *Lavandula* sp. and *Origanum* sp. are showed in previous studies. Essential oils major compounds of *L. angustifolia and L. x heterophylla* were identified as linalool, linalyl acetate, camphor, 1,8-cineole, and borneol. Camphor, α-fenchone, bornyl acetate, 1,8- cineole, and camphene were characterized and confirmed as major components of *L. stoechas* essential oil. In tested four different *Origanum* sp. EOs carvacrol was identified as the major component [17,18].

Bioautography analyses

The fingerprinting of EOs obtained from four *Origanum* and three *Lavandula* specimens was done by thin-layer chromatography. Visualization of the volatile components present in all EOs was performed by derivatization with Anisaldehyde reagent. The bioautography was performed for EOs with DPPH and ABTS methods. Clear zones at a same R_f value presented in Figures 1 and 2. The TLC–fingerprint analysis revealed that EO hydro-distilled from the aerial parts of *O. vulgare* (B) is the most abundant in chemical constituents. Carvacrol and linalool which main compounds of EOs were also visible in the EO obtained from the flowers (G); however, their abundance was different [19].

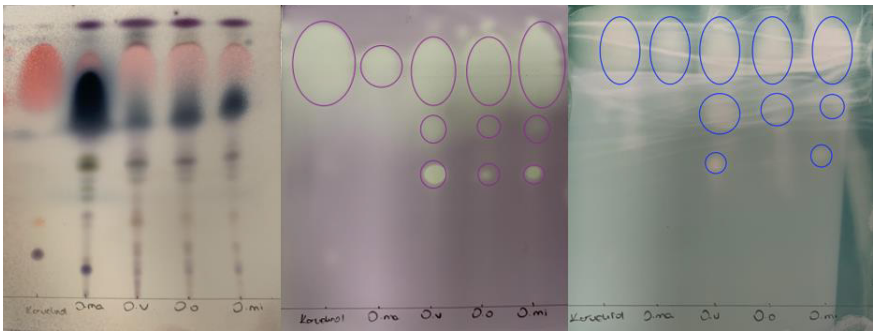

Figure 1. TLC-fingerprint, DPPH, and ABTS bioautography of studied material.
(From left to right: Carvacrol, *O. majorana, O. vulgare, O. onites, O. minutiflorum,* respectively)

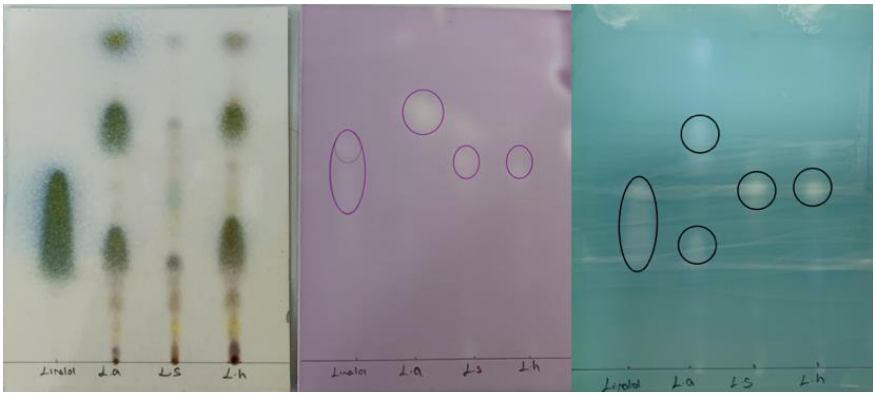

Figure 2. TLC-fingerprint, DPPH, and ABTS bioautography of studied material.
(From left to right: Linalool, *L. angustifolia, L. stoechas, L. x heterophylla,* respectively)

As shown in Figures 1 and 2, we can determine the major compounds of lavender and *Origanum* EOs are mostly responsible for antioxidant activity. By looking at the Rf values, we can indicate that carvacrol and linalool have significant antioxidant capacity and these compounds can be found in the tested EOs. However, in this assay, we can only understand the bioactivity of the compound that we've added to the TLC plate. In this case, we don't know the details about other active components. Further information on active components may be determined by different isolation techniques may be used such as PTLC, HPTLC, and GC-MS.

There are numerous studies have shown significant antioxidant activity results for different oregano and lavender species in the literature. Various essential oils such as clove oil, thyme oil, oregano oil, lavender oil, eucalyptus oil, pep-

permint oil, etc. play an important role in the inhibition of pathogenic microbial growth and food preservation [23–25]. Moreover, it is reported that twenty-one phenolic compounds isolated from *O. vulgare* ethanolic extract evaluated *in vitro* antioxidant activity using DPPH radical-scavenging and ferric-reducing antioxidant power (FRAP) assays[26]. Ethyl acetate, *n*-butanol, and water extracts of *O. vulgare* possessed a strong, and *O. majorana* showed moderate antioxidant activity in accordance with its phenolic compounds [27,28]. The importance of major compounds of *Origanum* essential oils is also indicated in various studies. Thymol and carvacrol showed high antioxidant activity according to different methods [29,30].

Studies indicates that linalool, camphor, and 1,8-cineole are the major constituent for *Lavandula* EOs. It is stated that, various *Lavandula* species has antioxidant activity due to their chemical composition [31–33]. Furthermore, linalool as itself showed antioxidant activity. The antioxidant activity from different cultivars affects environmental conditions, thus, it is expected to see different results in different samples. Some EOs of *Lavandula* plants were reported to have antioxidant properties, while some have none[33]**.** The bioautography results showed that there is antioxidant activity in *L. stoechas* and *L x heterophylla* due to linalool. However, in *L. angustifolia*, there are two different spots that we couldn't determine, has a significant antioxidant capacity as well.

Antimicrobial studies

Due to the development of antibiotic resistant microorganism and urge to find new antibacterial agents, essential oils are being evaluated as excellent resources to inhibit the resistant microorganisms. Therefore, lavender and *Origanum* essential oils were evaluated for their antibacterial activities (Table 1 and 2) by determining MIC.

Table 1. MIC values of amoxicillin, tetracycline and *Origanum* essential oils in µg/mL by broth microdilution assay

Material	*O. majorana*	*O. vulgare*	*O. onites*	*O. minutiflorum*	Amoxicillin
Methicillin Resistant *Staphylococcus aureus* (MRSA)	125	62.5	62.5	125	> 1000
Streptococcus mutans	15	7	3.5	1.75	125

Table 2. MIC values of amoxicillin, tetracycline and *Lavandula* essential oils in µg/mL by broth microdilution assay

Material	*L. angustifolia*	*L. stoechas*	*L. x heterophylla*	Amoxicillin
Methicillin Resistant *Staphylococcus aureus* (MRSA)	125	250	125	> 1000
Streptococcus mutans	250	62.5	125	125

Previous studies showed that *Origanum* essential oils have an antibacterial effect. *O. vulgare* essential oil showed strong antibacterial activity against the *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Bacillus cereus,* and *Candida albicans* strains. Costa et al. (2009) have reported the antibacterial activity of oregano oil against *E. coli, Enterococcus faecalis,* and Meticillin-resistant *Staphylococcus aureus* (MRSA) [34]. In several studies the major constituents such as thymol, carvacrol, eugenol, linalool and α-terpineol possess antimicrobial properties. Furthermore, linalool as itself showed antibacterial activity against different strains such as *P. aeruginosa* [10]. In controversy, some studies have shown *L. stoechas* has no activity against various pathogens such as *S. aereus, S. epidemidis, E. coli,* and *P. aeruginosa,* however, found active against *Salmonella Typhimurium, and Klebsiella pneumoniae*[33]. According to our results*, L. angustifolia, L. x heterophylla, O. majorona,* and *O. minutiflorum* showed moderate inhibition to MRSA, and *O. vulgare* and *O.onites* showed remarkable inhibition activity. Additionally, all tested *Origanum* essential oils shown inhibitory activity against *S.* mutans.

In this study, we determine the antioxidant activity of commonly used four different *Origanum* and three different *Lavandula* essential oils via bioautography assay to evaluate the major antioxidant capacity of the volatile compounds. In addition to the bioautography assay, we investigated the antibacterial activity against two different pathogens which variously affect a person's health. Between the tested essential oils, *O. minutiflorum* showed the highest activity compared to other oregano EOs, and *L. stoechas* showed the highest activity compared to tested lavender species, against *S. mutans* and most of the tested essential oils had better activity compared to Amoxicillin. Moreover, *O. onites* and *O. vulgare* showed the highest activity against MRSA and all the tested essential oils showed better inhibitory activity against MRSA, compared to the tested antibiotic. Other tested *Lavandula* and *Origanum* EOs also

showed high antibacterial activity against MRSA compared to Amoxicillin. The results of the bioautography assay highlighted that the antioxidant activity was majorly caused by the linalool and carvacrol. However, other undefined spots also have significant antioxidant activity. These antibacterial and antioxidant activities may be related to the complexity of volatile constituents, and bioautography assay also indicated this assumption. Previous studies showed the major components of EOs and their biological activity. Antimicrobial, antioxidant, and insecticide activities proved for linalool and carvacrol. Essential oils and their major constituents, effectively enhance the safety and quality of food products, due to their antimicrobial and antioxidant activity capacities[23]. With the bioautography screening of antioxidant compounds and the antimicrobial assay led to the identification of carvacrol and linalool as the major antioxidant constituent of the tested essential oils. The results obtained indicate that oregano and lavender essential oils are a good source of natural antioxidants with potential application in food and pharmaceutical industries, and a good antibacterial agent, they can be a safer alternative to synthetic agents.

As further studies, we aim to investigate the undefined antioxidant constituents and will try to match them with the GC-MS analyses.

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