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Antibacterial activities of *Melissa officinalis* (Lamiaceae) aerial parts extracts against bacteria isolated from the oral microflora, and their antioxidant properties

Research Article

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Abstract

Dental caries and periodontal diseases are common oral conditions associated with the formation of dental plaque. Dental plaque is composed of the oral microbiota and accumulates on the tooth surface. Despite the development of numerous antiseptic agents to control bacterial growth, the issue of bacterial resistance has prompted scientists to explore new avenues. As the problem of bacterial resistance continues to grow, studies in this field become increasingly crucial. Medicinal plants offer the best resources for this purpose, and *Melissa officinalis* L. is one such plant. This study aims to determine the antibacterial and antioxidant activities of *Melissa officinalis* against bacteria isolated from the oral microbiota. In the study, 8 bacteria were used as the organism source, and *Melissa officinalis* was utilized as the plant source. The extraction of the plant was carried out using ethanol, methanol, and water. All extracts were evaluated for their antibacterial activities using the disk diffusion method. Antioxidant activity studies were conducted using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method. At the end of the study, the highest antibacterial activity was obtained from the ethanol extract of *Melissa officinalis*, with a 26 mm inhibition zone diameter against *Staphylococcus epidermidis* MBKK7. The highest antioxidant activity was determined to be 87.34% DPPH radical scavenging activity from the methanol extract of *Melissa officinalis*. As a result, it has been determined that *Melissa officinalis* exhibits both high antibacterial and antioxidant activities. It can be suggested that *M. officinalis* may serve as an effective natural protector against oral pathogens.

Keywords: Antibacterial activity, Antioxidant activity*, Melissa, Serratia, Staphylococcus.*.

Introduction

Periodontal diseases and dental caries are prevalent oral conditions among the human population, influenced by various factors and strongly associated with the buildup of dental plaque. Dental plaque consists of the natural oral microbiota and accumulates on the surface of teeth. To control bacterial growth, numerous antiseptic agents are commonly employed (1, 2). Nevertheless, it's important to note that these substances can have negative side effects. The primary approach to addressing microbial infections revolves around the widespread use of conventional therapeutic antibiotics. However, the overuse of these antibiotics has led to a significant increase in resistance among pathogenic microorganisms. Additionally, these common therapeutic antibiotics often come with drawbacks such as hypersensitivity, immune suppression, and allergic reactions (3, 4, 5).

The effectiveness of numerous antibiotics is diminishing, primarily due to the emergence of antibiotic resistance resulting from their overuse. Antibiotic resistance mechanisms primarily involve the expulsion of antibiotics by transporters and hindrance of antibiotic interaction with their intended targets through mutation, modification, and target safeguarding. These mechanisms can be attributed to inherent structural or functional resistant traits, acquired resistance through mutational changes or horizontal gene transfer, as well as adaptive antibiotic resistance (6).

Studies indicate that if the current trend of antibiotic resistance persists, it could lead to over 10 million annual human deaths from antibiotic-resistant infections by 2050, with an estimated global economic burden of $100 trillion (7). Consequently, there is a pressing need for comprehensive research efforts to develop new antimicrobial agents that demonstrate high effectiveness against a broad spectrum of bacteria.

As per the World Health Organization (WHO), medicinal plants are considered an ideal source for a diverse range of pharmaceuticals (8). The World Health Organization has revealed that approximately 80% of the global population continues to depend on herbal remedies for their healthcare requirements (9). This reliance stems from the perceived safety of herbal medicine, which is associated with fewer side effects compared to synthetic drugs (10). Over the past decade, there has been a notable upsurge in research concerning herbal medicines and their bioactive constituents (11). Nevertheless, there is a significant dearth of information

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regarding such activities of medicinal plants. Out of the approximately 400,000 plant species on Earth, only a limited subset has been systematically examined for their antimicrobial properties (12).

As a result, exploring the antimicrobial properties of medicinal herbs has emerged as a natural alternative to conventional chemical antibiotics for infection treatment (3, 4, 5). Medicinal plants stand as primary reservoirs of natural antimicrobial and antioxidant compounds. As per the World Health Organization, medicinal plants are considered a prime source for a wide spectrum of drugs. Consequently, it is imperative to conduct thorough investigations into these plants to gain a deeper understanding of their properties, safety, and effectiveness (13). It is known that there are many medicinal and aromatic plants that have an impact on human health. Most of these are not well known to the public. One of these medicinal and aromatic plants is *Melissa officinalis* L.

Melissa officinalis L., which is commonly referred to as lemon balm, honey balm, balm mint, garden balm, or common balm, is a perennial herbaceous plant belonging to the Lamiaceae family. It has three subspecies: *Melissa officinalis* subsp. *officinalis*, *Melissa officinalis* subsp. *altissima*, and *Melissa officinalis* subsp. *inodora*. The dried leaves of *Melissa officinalis* are used to make herbal tea (14). However, both dried and fresh leaves and aerial parts of the plant are utilized in medicine, food, and cosmetics (15, 16, 17). The Lamiaceae family comprises approximately 236 genera and around 6900-7200 species.

It is primarily found in the Mediterranean region and can also be located in regions such as Central Asia, Iran, Europe, Serbia, the Americas, and Africa. *M. officinalis* has the characteristic of rapid growth. When fully matured, the plant typically reaches an average height ranging from 70 to 150 cm (18, 19, 20). This plant is found in various provinces in Turkey, including Istanbul, Ankara, Bursa, Bilecik, Bolu, Amasya, Samsun, Kütahya, Malatya, and Tunceli (21). The leaves of *Melissa officinalis* L. are characterized by their dark green color and ovate shape, emitting a subtle lemon fragrance reminiscent of mint. During the summer, the plant produces small white flowers brimming with nectar, which make it an attraction for bees (22). The medicinal attributes of *M. officinalis* extract have made it a staple in the traditional medicine systems of many countries for the treatment of various ailments (23, 24, 25, 26, 27, 28).

Scientific research has established that the therapeutic properties of *M. officinalis* are attributed to a diverse array of secondary metabolites, including flavonoids, phenolic acids, and terpenes (20, 29, 30, 31). Most of these secondary metabolites are derived from its essential oil, which contains compounds such as eugenol, octinol, octin, octinone, citral, hexenol, and haramin. Additionally, with a notable rosmarinic acid content of 36.5 per gram of the plant, it finds applications in the treatment of various diseases (19).

The essential oil and extracts derived from *M. officinalis* have been demonstrated to possess antibacterial, antiviral, antifungal, antioxidant, anti-inflammatory, antinociceptive, and antidiabetic effects. Additionally, it is reported to be utilized as an anxiolytic, antidepressant, anti-stress, anti-Alzheimer, and neuroprotective agent, providing protection against cardiovascular diseases due to its hypotensive property. The essential oil from *Melissa* is used to alleviate indigestion, bloating, colic, and nausea (32). These effects are primarily attributed to bioactive compounds present in the extracts and volatile oil. Studies indicate that the biological activities are predominantly attributed to compounds such as rosmarinic acid, caffeic acid, p-coumaric acid, and ferulic acid (16, 17, 32, 33, 34, 35, 36, 37, 38).

Living organisms deploy an antioxidant defense system against free radicals to mitigate potential damage. Dysfunctional antioxidant systems may lead to an accumulation of free radicals, accelerating aging, causing cell death, tissue damage, and even cerebral hemorrhage (39). Antioxidants intervene by either inhibiting the formation of free radicals or scavenging existing ones, thereby preventing cell damage and thwarting degenerative diseases (40, 41). The antioxidant properties found in the phenolic compounds of *M. officinalis* leaves are attributed to their redox properties, allowing them to function as effective reducing agents. Consequently, these antioxidants neutralize free radicals within cells, mitigating cellular damage and providing an anti-genotoxic effect (19).

The antioxidant properties of *Melissa officinalis* L. are primarily attributed to its phenolic compounds and flavonoids (42, 43, 44, 45, 46, 47, 48, 49). These antioxidants effectively neutralize both natural and synthetic free radicals, implicated in various health issues such as cardiovascular diseases, cancer, skin problems (49), and Alzheimer's disease. The development of these diseases is often associated with the detrimental effects of free radicals on essential biomolecules like DNA, proteins, and lipids (50). While synthetic antioxidants like BHA (butylated hydroxyanisole) and BHT (butylated hydroxytoluene) were previously prevalent in food preservation, concerns over their potential toxicity and carcinogenic properties have shifted attention towards natural sources of antioxidants (49).

Medicinal plants rich in polyphenols have emerged as a significant natural source of antioxidants. The antioxidant activities of these compounds are attributed to their ability to chelate iron (II), reduce DPPH (a stable free radical), scavenge free radicals, inhibit the generation of reactive species that trigger lipid peroxidation, and regulate the plasma concentration of enzymes involved in the body's defense against reactive oxygen species (20). As a result, the pursuit of novel, safe, and effective antioxidants derived from natural sources has emerged as a predominant area of research (51).

The World Health Organization (WHO) stated that *Escherichia coli* and *Staphylococcus aureus* are the priority pathogens for overcoming antimicrobial resistance and for the research and development of new antibiotics. In the studies conducted to date, no studies

have been found on the biological activities of *Melissa officinalis* against oral pathogens. In this study, our primary focus was to assess the antioxidant activity of extracts obtained from the *Melissa officinalis* plant and their antimicrobial effects against oral pathogens isolated from the mouth. Notably, this investigation marks the first instance of evaluating the antibacterial aspects of *Melissa officinalis.*

Materials and Methods

Organisms

The test microorganisms for antimicrobial activity were provided from the previous studies of Prof. Dr. Gulten Okmen, totalling 8 strains. All organisms were cultured at 37 ˚C for 24 hours on plates containing Nutrient Broth (NB) (Merck).

Plant material

In this study, the plant *M. officinalis* L. belonging to the Lamiaceae family was utilised. The plant material was diagnosed by Dr. Olcay Ceylan and is preserved in the herbarium of Muğla Sıtkı Koçman University, Department of Biology, Turkey. The plant used in the study was obtained from herbalists in Mugla in the year 2017 (52).

Cultivation of organisms

The bacteria used in the antimicrobial activity studies were aseptically cultured in liquid nutrient media containing Nutrient Broth (NB) (Merck) and were incubated at 37 ± 0.1 ˚C for 24 hours using an incubator (Nüve EN 400).

Plant extraction

The plants were mechanically pulverised into powder using a blender (Fakir). All materials were stored at room temperature in a light-shielded area until sample preparation, and subsequently kept at 4 ˚C until needed for analysis. Dried and powdered plant organs were weighed to 50 g, and these plant samples were placed in Erlenmeyer flasks for water extraction with the addition of distilled water (250 mL) for a period of 6 to 8 hours. For ethanol and methanol extraction, they were placed in a Soxhlet apparatus (Isotex) and separately extracted with methanol and ethanol solvents (250 mL) for 8 to 10 hours. The solvent-evaporated extracts were transferred into sterile Falcon tubes, each in its own solvent, and stored in refrigerated conditions until use.

Determination of antibacterial activity

The antibacterial activity studies were conducted using the Kirby-Bauer method (53). The antibacterial activities of plant extracts were tested against microorganisms isolated from the oral flora. Pure cultures were incubated at 37 ˚C for 24 hours in Mueller Hinton Broth (MHB), and the turbidity of the cultures was adjusted to 0.5 McFarland ($1.5x10^8$ cfu/mL). Then, 0.1 mL of active bacterial cultures was individually inoculated onto Mueller Hinton Agar (MHA) medium. After aseptically spreading, blank discs (6 mm) (Bioanalyse) were impregnated with 25 µL of plant extracts and placed on the surface of the agar plates. After incubation at 37 ˚C for 24 hours, the plates were examined, and the inhibition zone diameters were measured and recorded in millimeters. As negative controls, water, ethanol, and methanol were used, while amikacin (30 µg), nalidixic acid (30 µg), novobiocin (30 µg), and oxacillin (5 µg) antibiotics were employed as positive controls. Each experiment was conducted in triplicate, and the results were presented as the mean values.

Determination of minimum inhibitory concentrations (MIC)

The minimum inhibitory concentration (MIC) studies were conducted using the broth dilution method. In this study, the MIC values of plant extracts were determined. The MIC value was considered as the lowest concentration that inhibited the growth of oral pathogens after incubation. The concentrations of the active cultures used in the experiments were adjusted according to 0.5 McFarland. All experiments were conducted in 2 mL Mueller-Hinton Broth (Merck) medium. To determine the minimum inhibitory concentration of plant extracts exhibiting antibacterial activity, serial dilutions were prepared at concentrations of 6500, 3250, 1625, and 812.5 µg/mL, and equal amounts (100 µL) of active bacterial cultures were inoculated into each dilution. All experiments were incubated at 37 ˚C for 24 hours, and after this period, MIC values were determined (54, 55, 56).

Determination of *in vitro* antioxidant activity

The antioxidant activities of plant extracts were determined by measuring their capacity to scavenge DPPH radicals. In the studies, a solution of methanolic DPPH (0.004 g) radical in methanol (100 mL) was utilised. Extracts (0.1 mL) were mixed with 2.9 mL of methanolic DPPH solution (0.1 mM) and left in the dark for 30 minutes. After the incubation period, the absorbances of the extracts were measured spectrophotometrically at 515 nm (Optizen). Methanolic DPPH was used as a control, and trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) served as a standard antioxidant (57). The percentages of DPPH radical scavenging for the samples were determined using the formula based on the obtained absorbance values.

Preparation of the Trolox standard curve

Trolox was used as a standard in radical scavenging activities, and the results were expressed in terms of mM Trolox equivalent (TE). To draw the Trolox standard curve, solutions of 2.5 mM Trolox were prepared in ethanol or 5 mM phosphate buffer saline (pH 7.4) (0.25; 0.5; 1; 1.5; 2; 2.5 mM), and absorbance values were measured (58). The absorbance values for the study were calculated based on the formula derived from the standard curve.

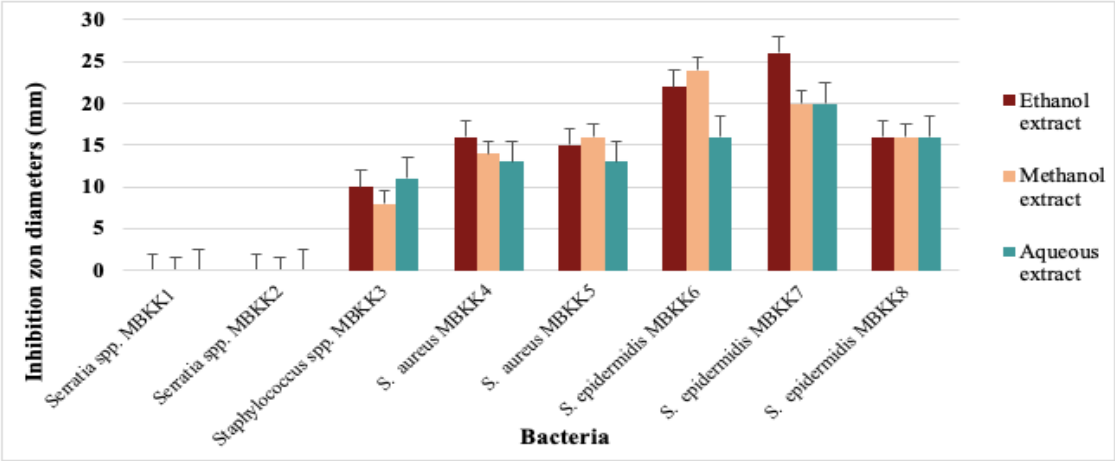
Statistical analysis

In this study, the means of the activities were calculated with excel 2016.

Results

When examining the results of antibacterial activity, it was observed that the extracts affected the bacteria differently. The highest antibacterial activity was recorded with a 26 mm inhibition zone diameter against *Staphylococcus epidermidis* MBKK7 in the *Melissa officinalis* ethanol extract. In the *Melissa officinalis* methanol extract, a 24 mm zone diameter was found against *Staphylococcus epidermidis* MBKK6. In the *Melissa officinalis* aqueous extract, the highest activity was determined with a 20 mm zone diameter against *Staphylococcus epidermidis* MBKK7. None of the *Melissa officinalis* extracts showed activity against *Serratia* spp. (Figure 1).

Figure 1: Antibacterial activities of *Melissa officinalis* extracts against oral pathogens



EE: Ethanol extract; ME: Methanol extract; AE: Aqueous extract; (-): No Inhibition

The antibiotic sensitivities of bacteria were tested with different antibiotics, and the results are presented in Table 1. Another study is the determination of the Minimum Inhibitory Concentration (MIC). In this study, the lowest MIC value was determined to be 3250 µg/mL in *Melissa officinalis* ethanol extract. Additionally, a MIC value of 3250 µg/mL was also determined for *Staphylococcus* spp. MBKK3 in the methanol extract (Table 2). Another biological activity test is the antioxidant activity, and in this study, DPPH radical scavenging activity has been determined. Considering the study data, the highest radical scavenging activity was obtained with 87.34% from *Melissa officinalis* methanol extract, while the lowest radical scavenging activity was determined to be 62.27% from *M. officinalis* ethanol extract (Table 3).

Table 1: Susceptibility of oral pathogens to standard antibiotics

Bacteria	Standard antibiotics	Inhibition zone diameters (mm)
Serratia spp. MBKK1	Amikacin	21
Serratia spp. MBKK2	Nalidixic acid	22
Staphylococcus spp. MBKK3	Oxacillin	9
Staphylococcus aureus MBKK4	Novobiocin	28
Staphylococcus aureus MBKK5	Novobiocin	18
Staphylococcus epidermidis MBKK6	Novobiocin	40
Staphylococcus epidermidis MBKK7	Novobiocin	38
Staphylococcus epidermidis MBKK8	Novobiocin	36

Table 2: Minimum inhibitory concentrations of *Melissa officinalis* extracts against oral pathogens (µg/mL)

Bacteria	Extracts		
	ME	EE	AE
Serratia spp. MBKK1	NT	NT	NT
Serratia spp. MBKK2	NT	NT	NT
Staphylococcus spp. MBKK3	3250	3250	(-)
Staphylococcus aureus MBKK4	(-)	3250	(-)
Staphylococcus aureus MBKK5	6500	3250	(-)
Staphylococcus epidermidis MBKK6	6500	3250	(-)
Staphylococcus epidermidis MBKK7	6500	3250	(-)
Staphylococcus epidermidis MBKK8	6500	3250	(-)

EE: Ethanol extract, ME: Methanol extract, AE: Aqueous extract, NT: Not tested, (-): The MIC value was found to be above the experimental concentrations.

Table 3: DPPH radical scavenging activity of *Melissa officinalis* extracts (%)

Activity	EE	ME	AE
% Inhibition	62.3	87.3	70.98
Trolox equivalent (TE)	1.8	2.35	2.01

TE: mM/g DW; DW: Dry weight; EE: Ethanol extract; ME: Methanol extract; AE: Aqueous extract

Discussion

Plant antimicrobial agents exert their effects by targeting specific regions of bacteria, fungi, and parasites. Among the primary mechanisms of antimicrobial compounds are the inhibition of protein synthesis, interference with cell wall synthesis, inhibition of metabolic pathways, interference with nucleic acid synthesis, and disruption of the cytoplasmic membrane, which are crucial pathways for implementing antimicrobial effects. Numerous essential oils derived from herbs derive their antimicrobial properties from the presence of compounds such as alkaloids, tannins, glycosides, flavones, anthraquinones, flavonoids, terpenes, anthocyanins, phenolics, and aldehydes. These bioactive substances are typically distributed across various parts of the herbs, including leaves, roots, shoots, blossoms, flowers, and barks. Aromatic plants, in general, are known for their richness in essential oils, which often exhibit significant antimicrobial properties. Gas Chromatography-Mass Spectrometry (GC-MS) analysis of the essential oils extracted from *Melissa officinalis* revealed a chemical composition dominated by citronellal (37.3%), thymol (11.9%), citral (10.1%), and β-caryophyllene (7.3%) (59).

Staphylococcus aureus bacteria are responsible for skin and subcutaneous infections, as well as foodborne illnesses. In this study, the highest antibacterial activity was recorded with a 26 mm inhibition zone diameter against *Staphylococcus epidermidis* MBKK7 in the *Melissa officinalis* ethanol extract. In addition, standard antibiotics were used in this study and the results of plant extracts and standard antibiotics were evaluated (Figure 1, Table 1). When reviewing the literature, it is evident that studies have investigated the antibacterial and antifungal activities of *Melissa officinalis* against various bacteria and fungi. However, this study marks the first investigation of its antibacterial activity against bacteria isolated from the oral microflora. In studies conducted until the present day, significantly different inhibition zone diameters have been reported for *S. aureus* and *S. epidermidis* (60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75). This study is consistent with the existing literature. These variations may be attributed to genotypic differences, climatic conditions, the use of different bacterial strains, variations in activity assays, and differences in extract concentrations (76). The inhibitory effect of *M. officinalis* against microorganisms can be attributed to its structural characteristics and its ability to modify and penetrate the lipid and protein structure of bacterial cell walls (77, 78, 79, 80, 81). The cell wall of Gram-positive bacteria is generally more susceptible to antimicrobial substances, chemical compounds, and herbal remedies. However, *Melissa officinalis* essential oil may penetrate the outer membrane lipopolysaccharides of bacteria directly due to the potential presence of alcohol-based compounds (19, 71).

In this study, Gram-negative bacteria were more resistant to extracts than Gram-positive bacteria (Figure 1). Divalent cations and the polysaccharide part of lipopolysaccharides in the outer cell membrane of Gram-negative bacteria confer hydrophilic qualities that hinder the contact of hydrophobic constituents (such as essential oils) with the bacterial cell. This leads to higher resistance of Gram-negative bacteria to the antibacterial properties of the essential oils (82, 83, 84, 85, 86).

In this study, the minimum inhibitory concentration (MIC) was found to be 3250 µg/mL in the ethanol extract of *Melissa officinalis* against *S. aureus* and *S. epidermidis* (Table 2). In the literature, MIC values of *M. officinalis* extracts vary. While some data align with the findings of this study, other studies report MIC values as either lower or higher (59, 63, 68, 70, 87, 88, 89, 90, 91). The discrepancies in antimicrobial activities, in comparison to the reported findings, may stem from variations in geographical conditions, plant age, oil isolation methods, cultivar types, and seasonal factors (92). Additionally, differences in the main and/or minor components of the oils could also contribute to these variations. Various factors such as climate, seasonal changes, and geographical conditions may influence the chemical composition of oils (93, 94).

The excessive generation of free radicals has been observed to inflict damage on biological materials, contributing to numerous physiological and pathological abnormalities, which are pivotal events in the etiopathogenesis of various diseases (95, 96, 97, 98). The DPPH scavenging assay results for plant extracts are presented in Table 3. The methanol extract of *Melissa officinalis* exhibited 87.34% inhibition (Table 3). These findings indicate that methanol proved to be the most effective solvent for extracting DPPH radical scavenging components from the plant samples. When examined in the literature, the reported data on the DPPH scavenging activities of *M. officinalis* show variations (60, 66, 63, 73, 99, 100, 101). The variations in antioxidant activities compared to the reported results may stem from differences in the methodologies employed or the geographical environment, cultivar type, seasonality, region's climate, physiological age of the plant, the plant species, maturity stage, harvesting stages, harvesting hours, drying methods, and the oil isolation technique (92, 93, 102, 103).

Conclusion

In this study, *Melissa officinalis* extracts have been found to possess noteworthy antioxidant activity. Therefore, in addition to being used as natural

preservatives in the food industry and oral hygiene, they could also be considered for use as natural remedies in infectious diseases. The current study reveals that all extracts of *M. officinalis* exhibited inhibitory effects on the tested *S. aureus* bacteria, while they were not effective against *Serratia* species. Future research should focus on determining the phytochemical composition of *M. officinalis*, conducting chemical analyses, and testing its efficacy against different bacteria in further *in vitro* studies.

Conflicts of interest

The authors state that there is no conflict of interest concerning the publication of this document.

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