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The Antibacterial Activities of *Lavandula angustifolia* L., *Mentha piperita* L., and *Ribes nigrum* L. against Oral Bacteria, and Their Antioxidant Activities

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ABSTRACT

There is an expanding interest in medicinal and aromatic plants as a natural alternative to synthetic drugs, especially to antimicrobial agents due to the growing problem of antibiotic resistance. In recent years, a lot of reports have been published on the antimicrobial activity of the plant extracts. This study was used *Lavandula angustifolia* L., *Mentha piperita* L., and *Ribes nigrum* L. as plant materials. This study aims to test the plant extracts against oral bacteria. Its purpose is to produce directly comparable, quantitative, antimicrobial data, and in addition to containing very little information of the different extracts against oral pathogens. Disc diffusion method was studied for antimicrobial activity tests. Also, minimum inhibitory concentration (MIC) values were determined in this study. Additionally, the extracts were tested against stable 2,2-diphenyl-1-picryl-hydrazyl-hydrate• (DPPH•) free radicals for non-enzymatic antioxidant activity. This study was used Trolox (6-hydroxy-2,5,7,8-tetra-methyl chroman-2-carboxylic acid) as standard. The extracts showed different inhibition zones against bacteria. The methanol extract of *Lavandula* showed the highest inhibition zone against the oral pathogen MBKK5. The positive control was penicillin (10 µg). The lowest MIC value was taken at 6500 µg /ml concentration of the plant extracts. The highest DPPH• radical scavenging activity was found in *Ribes nigrum* extract as 36%. As a result, plant extracts have antibacterial and antioxidant potential.

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Introduction

Mouth diseases continue to present a serious health problem worldwide (Petersen et al., 2003; Akgül et al., 2022). According to the World Health Organization (WHO), the oral disease burden is a major international health concern in the 21st century (Mak and Dey, 2011). The periodontal diseases and dental caries are among the most important global oral health problems. Worldwide, dental caries causes pain and discomfort in almost 100% of adults and 60-90% of school-age children. In addition, 35-50% of middle-aged adults (ages 35-44) suffer from severe periodontal disease, which can result in tooth loss (WHO, 2012). According to the Global Burden of Disease Study 2017 estimated that oral diseases affect close to 3.5 billion people worldwide, with caries of permanent teeth being the most common condition. In the world, it is reported that 2.3 billion people pull from caries of permanent teeth and more than 530 million children suffer from caries of primary teeth (IHME, 2018). In the last years, due to the extreme use of antibiotics in the treatment of many diseases in developing countries, microorganisms have gained resistance to these drugs over time. This reduces the effectiveness of the conventional medication (Chung et al., 2006; Korkmaz et al., 2021). The chemical preservatives used in the prevention and treatment of oral diseases cause tooth staining and toxicity (Rodrigues et al., 2007). Therefore, phytochemical compounds produced by plants that are traditionally used against diseases are a good alternative to synthetic chemicals (Chitme et al., 2003; Sevindik et al., 2017; Uysal et al., 2021).

Lavandula L. is a Mediterranean plant, perennial herbaceous or aromatic shrub, belonging to the *Lamiaceae* family. It is known that *Lavandula angustifolia* species act an important role in the pharmacology and perfumery-cosmetics industry due to the essential-aromatic oil content of especially dark purple flowers and leaves (Seçmen et al., 1998). Among its active ingredients, linalool and linalyl acetate have a significant proportion (Uğur and Everest, 2017; Üstü and Uğurlu, 2019). There are three types of *Lavandula* in Türkiye. Of these, *L. angustifolia* subsp. *angustifolia* originates from the Western Mediterranean

and is known as medicinal lavender (*L. officinalis*), and is the most cultivated species due to its importance in the perfumery industry. The chemical composition of the essential oil obtained from the dried flowers of *Lavandula angustifolia* grown in Poland was determined using of GC, GC-MS and NIR analyzes. Seventy-eight compounds have been determined in the oil. It has been determined that the basic components of the oil are linalool (30.6%), linalyl acetate (14.2%), geraniol (5.3%), β-caryophyllene (4.7%), lavandulyl acetate (4.4%) (Smigielski et al., 2009).

Mentha (*Lamiaceae*) is one of the plants used as food for medicinal purposes, and they have pharmacological and cosmetic importance due to their menthol content (Aytaç and İğci, 2012). The leaves are simple, the flowers are hermaphrodite, and find as many flowers (Davis, 1965; Davis, 1988; Seçen et al., 1998). *Mentha piperita* L.; In English, it is the herb, also known as peppermint. It is one of about 10 mint species that naturally spread in our country and is also known as English mint or spearmint (Güner et al., 2012). *M. piperita* is the most economically important mint taxon, which oil is extremely popular because of menthol and menthone compounds (Ludwiczuk et al., 2016). Both the plant itself and its oil are used as both traditional and commercial medicinal plants in the treatment of diseases such as colds, throat and stomach problems and cancer all over the world (Singh et al., 2015; Çinbilgel et al., 2019). The chemical composition of the essential oil obtained from peppermint (*Mentha piperita* L.) was analyzed by GC/FID and GC-MS. While menthol (40.7%) and menthone (23.4%) were the main components, (+/-)-methyl acetate, 1,8-cineol, limonene, beta-pinene, and beta-caryophyllene were determined as other components (Schmidt et al., 2009).

Ribes is a member of the *Grossulariaceae* family. This plant is deciduous or rarely evergreen shrub, with or without thorns. Usually, the petals are smaller than sepals (Davis, 1965; Davis, 1988; Seçmen et al., 1998). The eight species of *Ribes* have been identified in the flora of Türkiye, and one of them is *Ribes nigrum*, known as "black currant" or with its new name "karagat". *Ribes nigrum* L., known as "blackcurrant" or "black currant" in English, is an unbranched, strongly aromatic shrub that is 1-2 m tall. The fruit is spherical, black, or rarely olive green (Davis, 1965; Davis, 1988). It is known that many parts of the plant, especially the fruit and leaves, are used in traditional treatment. For example, the leaves of the plant are used in the remedy of rheumatic diseases such as arthritis, in the elimination of respiratory and urinary system problems, and various injuries with insect bites (Kendir et al., 2016; Kendir et al., 2019). The extraction of phenolic compounds has been optimized for different parts of the *Ribes nigrum* (blackcurrant) plant and an efficient method has been developed for their separation by HPLC. A total of 23 compounds were detected in the buds, 22 of which were in the fruit and leaves. In addition, it has been reported as the first evidence of kaempferol-3-O rutinoside in black currant leaves (Vagiri et al., 2012).

In the study, the biological activities of ethanol, methanol, and aqueous extracts of 3 different plants against oral pathogens were investigated, and it was aimed to contribute to the little information in the literature about the antibacterial and antioxidant activities of these plant extracts. These plants are *Lavandula* (flower), *Ribes* (fruit), and *Mentha* (leave).

Materials and Methods

Chemicals and Reagents

All of the chemicals and reagents have analytical purity. These are including Methanol (Merck), Ethanol (Merck), Mueller-Hinton Broth (Merck), Mueller-Hinton Agar (Merck), 2,2-Diphenyl-1-picryl-hydrazyl-hydrate (DPPH, TCI), 6-Hydroxy-2,5,7,8-tetra-methyl chroman-2-carboxylic acid (Trolox, Merck), Penicillin (Bioanalysis, 10 μg).

Plant materials

The plant samples, which are the research material, were obtained from Mugla (C2) region and local herb shops. There are three plants used in the study; *Lavandula angustifolia* (flower), *Mentha piperita* (leave), *Ribes nigrum* (fruit). Plant materials were defined by Prof. Dr. M. Guven Gork. The diagnosis of plant materials was made according to Davis (1978). The plants were preserved at ambient temperature and darkroom until used for extraction. The plants have been hidden at the herbarium of Mugla Sitki Kocman University. Herbarium specimen numbers of the plants were O.1510, O.1511, and O.1512, respectively.

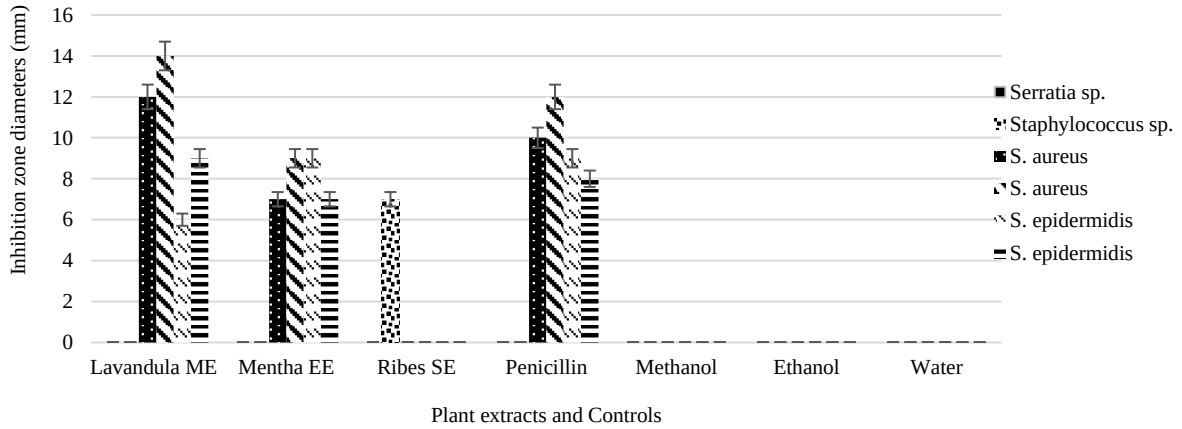

Figure1. Antibacterial activities of different plant extracts against oral bacteria (200 mg/mL)
ME: Methanol extract; EE: Ethanol extract; SE: Aqueous extract

Table 1. Minimum inhibitory concentrations of extracts of different plants against oral pathogens

Bacteria	Plant extracts (μg. mL^{-1})		
	Lavandula (ME)	*Mentha* (EE)	*Ribes* (SE)
Staphylococcus sp. MBKK 3	(-)	(-)	nd
S. aureus MBKK 4	13000	6500	(-)
S. aureus MBKK 5	13000	13000	(-)
S. epidermidis MBKK 6	13000	6500	(-)
S. epidermidis MBKK 7	6500	6500	(-)

ME: Methanol extract; EE: Ethanol extract; SE: Aqueous extract; (nt): Not tested; (-): inhibition did not occur

Table 2. DPPH radical scavenging activities of plants

Plant extracts (200 mg. mL^{-1})	Scavenging activity (%)
L. angustifolia	21.20
M. piperita	8.30
R. nigrum	36.70

Microorganisms

There are six bacteria used in this study, these are; *Serratia* sp. MBKK2, *Staphylococcus* sp. MBKK3, *S. aureus* MBKK4, *S. aureus* MBKK5, *Staphylococcus epidermidis* MBKK6, *S. epidermidis* MBKK7. Bacterial cultures waited in Mueller-Hinton Broth (Merck) medium at 37°C for 24 hours. The bacteria were obtained from Assoc. Dr. Gulten Okmen's previous works. All of the bacteria were deposited at Microbial Biotechnology Culture Collection (MBKK) in Türkiye.

Preparation of Plant Materials

The samples were washed 2-3 times in running water and once in sterile distilled water. The plants were shade-dried at room temperature (37±1°C) for a week. The plants were pulverized in a blender (Arzum, Mio). All of the materials were stored at room temperature until sample preparation, then stored at 4°C until (Arcelik, Türkiye) needed for analysis. These plants were passed through a flour sieve before use for extraction.

Preparation of Plant Extracts

The air-dried and powdered plants were extracted with solvents using soxhlet. These solvents were methanol, ethanol, and water. After evaporation of the extracts in organic solvents, each of them was stored in its own solvent in sterile opaque bottles under refrigerator conditions until used. Evaporation was done at 70°C using the rotary evaporator (Heidolph, WB200)

Determination of in vitro Antibacterial Activity

The antibacterial activity studies were performed using the Bauer-Kirby (1966) method. The plant extracts (200 mg. mL^{-1}) were tested by disk diffusion method, The cultures were incubated on Mueller-Hinton Agar plates (MHA, Merck) for 24 hours at their own temperature. The turbidity of bacterial cultures was adjusted 0.5 McFarland. After incubation, the inhibition zones formed were recorded in mm. In the study, the reference antibiotic used as a positive control was penicillin (10 μg).

***Determination of Minimum Inhibitory Concentration* (MIC)**

In the study, the values of the minimum inhibitory concentration of the extracts as antibacterial activity were also determined. MIC value, inhibit growth after incubation was taken as the lowest concentration of extract. The broth dilution method has been tested as defined in the CLSI standards (CLSI, 2003; CLSI, 2006). The final concentrations of each extract in this test were adjusted to be 13000, 6500, 3250, 1625, 812.5, and 406.25 μg. mL^{-1}.

Determination of Antioxidant Activity

In non-enzymatic antioxidant activity studies, 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH$^{\bullet}$) was used as a free radical. The DPPH method was used to determine the free radical scavenging activity of the extracts. 0.1 mL of the extract was added to 2.9 mL of methanol DPPH solution (0.1 mM). The extracts were incubated for 30 minutes, and then their absorbances were measured at 515 nm. DPPH solution with methanol was used as control and methanol was used as blank. 6-Hydroxy-2,5,7,8-tetra-methyl chroman-2-carboxylic acid (trolox) was used as the reference antioxidant. DPPH scavenging capacities were calculated using the formula and given in % (Brand-Williams, 1995).

Statistical Analysis

All of the experiments were conducted in triplicate and the data are presented as mean values ± standard deviation. Analysis of standard deviation and means were made with the Microsoft Excel 2016 program.

Results and Discussion

In literature, there are studies with *Lavandula*, *Mentha*, and *Ribes*. But most of the studies are experimented with against common pathogens and viruses. There are a few studies against oral pathogens in the literature. However, standard strains were used in these studies, and the bacteria were not isolated from mouth flora. There are two studies of *Lavandula* in literature. At the end of these studies, researchers found MIC value as 1 μL for *S. aureus* (Thosar et al., 2013). In another study determined MIC value was 0.31 μg.mL^{-1} for *S. aureus* (Rapper et al., 2016). There are three studies of *Mentha* in literature. Raghavan et al (2018) reported that the inhibition zone was found as 20 mm against *Streptococcus* mutans. Pramila et al. (2012) reported that they were determined 1.10 mm for *Staphylococcus* sp. In another study, researchers reported 2.33 mm against *S. aureus* (Horvath and Koscova, 2017).

At the end of this study, it was observed that *L. angustifolia* methanol and *M. piperita* ethanol extracts showed antibacterial activity against *S. aureus* MBKK4, *S. aureus* MBKK5, *S. epidermidis* MBKK6, and *S. epidermidis* MBKK7. It was determined that water extract of *R. nigrum* has antibacterial activity against one oral pathogen (7 mm). This bacterium is *Staphylococcus* sp. MBKK3. As a result, when all plant extracts were examined, the highest antibacterial activity belonged to the methanol extract of *L. angustifolia*. The inhibition zone diameter of this plant extract against *S. aureus* MBKK5 was 14 mm. This value obtained from plant extract was found to be higher than the inhibition zones of penicillin (Figure 1).

Another antimicrobial activity test is MIC. Table 1 contains the MIC values of different solvents of 3 plants. MIC tests were only studied for plants having a zone of inhibition against bacteria. According to the results of the broth dilution method, the lowest MIC value was obtained from *M. piperita* ethanol extract (6500 $\mu g.mL^{-1}$) against *S. aureus* MBKK4, *S. epidermidis* MBKK6, and *S. epidermidis* MBKK7 bacteria. Additionally, the ethanol extract of *L. angustifolia* was also determined to have the lowest MIC value (6500 $\mu g.mL^{-1}$) against *S. epidermidis* MBKK7 bacteria (Table 1). In addition, the minimum inhibitory concentrations in *R. nigrum* aqueous extract were not determined at any of the concentrations tested.

Antioxidant activity studies were applied to all of the plants, and the data obtained are given in Table 2. According to the results of this study; radical scavenging activities were for *L. angustifolia* at 21%, *M. piperita* for at 36.7%, and *R. nigrum* at 8.3% (Table 2).

The medicinal use of the plants offers alternative solutions for the treatment of diseases. In the study, the different plant extracts were tested against six oral bacteria, and the antibacterial activities were compared with penicillin. In the study, the extracts of different plants showed antibacterial activities against four bacteria and did not show any activity against *Serratia* sp. MBKK2 and *Staphylococcus* sp. MBKK3 (Figure 1).

Prusinowska et al. (2016) tested the *L. angustifolia* plant against two bacteria and reported low antimicrobial activity and low DPPH scavenging activity (3.6-3.8%). As a result of this study, *L. angustifolia* showed both high antibacterial activity and high antioxidant activity (Figure 1, Table 2). Danh et al. (2013) were investigated the antimicrobial activities of 3 extracts of *L. angustifolia* against various microorganisms and their antioxidant capacities. As a result of the study, they determined that the highest inhibition zone diameter (28 mm) had been shown against *S. aureus*. Also, they reported DPPH scavenging activity as 63%. The data obtained from this study were found higher than our study. Akgul et al. (2022) reported that the free radical scavenging activity of *Euphorbia eriophora* ethanol extract was measured by the DPPH method. At the end of the study, they assigned that the DPPH activity of the plant extract had an inhibition value of 68.7 %. Sevindik et al. (2017) searched for the antioxidant, antimicrobial activities and oxidative stress properties of ethanol extracts of *Mentha longifolia* that collected from different location of Gaziantep province. DPPH radical scavening activity was used for antioxidant assay and antimicrobial efficacy was tested on six different microorganisms. As a result, they observed that the antimicrobial activity was 50-800 µg/mL, while the antioxidant activity varied between 1.809-3.628 mmol/L.

Bayrak et al. (2017) were reported that *L. stoechas* has low antimicrobial activity and high antioxidant activity. Ergün et al. (2018) reported that *L. stoechas* has antibacterial and antioxidant activities. The reason for this the composition of the plant extracts can be attributed to various factors such as environmental factors, processing, cultivar, and post-harvest (Houston, 2005). In addition, İlkimen and Gülbandılar (2018) stated in their study that *L. stoechas*, Sokovic et al. (2010) stated that the *L. angustifolia* plant showed antibacterial and antioxidant properties.

Saeed and Tariq (2005) tested *M. piperita* root and leaf juice against eleven different Gram-negative bacteria, reported that it showed the highest inhibition zone in root and leaf (15 and 17 mm, respectively). The results of this study show better results compared to our study.

This study supports other works in literature (Priya et al., 2007; Rasooli et al., 2008; Singh et al., 2015; Okmen et al., 2017).

In the study by Krisch et al. (2014) they tested water and methanol extracts of the *Ribes nigrum* against various bacteria and reported a low inhibition zone (Krisch et al., 2014). However, better results were obtained in this study (Figure 1). Kendir et al. (2016) were investigated the antibacterial activities of leaf and branch extracts of different *Ribes* species against various bacteria and reported that they showed activity against *S. aureus*. As a result of another study, they reported that some *R. nigrum* fresh fruits inhibited the growth of Gram-negative and Gram-positive bacteria (Cavanagh et al., 2003). The results of the study support the results obtained from the literature.

Conclusion

Considering the results of the study, it was determined that three plants have antibacterial activity against oral bacteria. Plant extracts and natural products can contribute to the development of new drugs that can make a significant improvement in the management of various health disorders. The strong antibacterial activity was obtained from the plants used in the study, especially *Lavandula* methanol and *Mentha* ethanol extracts. In addition, the extracts of *R. nigrum* have the highest antioxidant activity. Our results show that these plants have antibacterial compounds that can be used in traditional medicine. Therefore, it is suggested that it can be used as an antibacterial and antioxidant agent against oral pathogens. In future studies, it is necessary to determine the components of plant extracts, to determine more activities of these components, as well as to conduct *in vitro* and *in vivo* studies. Promising compounds of these plants can be deduced by further testing, including experimental models and pharmacological applicability. However, more research is needed to identify the biologically active compounds of plants.

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Author's Contributions

The contribution of the authors is equal.

Statement of Conflict of Interest

Authors have declared no conflict of interest.

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