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Investigation of The Antibacterial Activities of Micromeria Congesta and Some Other Plant Extracts

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Abstract

With the Covid-19 pandemic, interest in plants and herbal products has increased day by day. The tendency of people towards natural treatment methods has led to more studies on plants and herbal products. The aim of our study was to determine the essential oils of Micromeria congesta, Rosmarinus officinalis L., Sideritis stricta, Artemisia absinthium and Melissa officinalis, which are used in the treatment of various diseases among the people, to evaluate the in vitro antimicrobial activity against Klebsiella pneumoniae, Escherichia coli, Salmonella Typhimurium, Proteus vulgaris, Staphylococcus aureus isolates and Corynebacterium pseudotuberculosis (ATCC 19410) standard strain. The essential oils of M. congesta and M. officinalis plants examined in our study were found to have antibacterial activity as much as reference antibiotics (Streptomycin, Amoxicillin) by disc diffusion method. It was observed that the essential oil of A. absinthium was effective against C. pseudotuberculosis bacteria, while the essential oils of other plants were not effective against the tested bacteria.

***Key words**: Micromeria congesta, medicinal plant, essential oil, antibacterial activity*

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Introduction

In the past, people first used plants to treat their diseases. The use of plants and herbal products for different purposes dates back to ancient times. In line with scientific studies and commercial purposes, plants are used in many fields such as phytotherapy, aromatherapy, cosmetics and food industry (1). The use of herbal products, which have become popular especially in recent years, in the treatment of various diseases continues to increase today as in the past (2). The intensive use of medicinal plants, which are considered to be natural and healthy, may also cause some negative effects. It is important to use these plants under the control of a doctor or specialist. While the increase in the use of antibiotics and the development of resistance in bacteria against antibiotics is increasing day by day, no such resistance development is observed in herbal products with antibacterial activity. This important feature further increases the importance of herbal products (3).

The use of plants and herbal products for therapeutic purposes varies according to the level of development of countries. In developing countries, 80% of people treat their illnesses with herbal products; in some countries in the Middle East, Asia and Africa, this rate rises to 95%. This rate is lower in developed countries. For example, this rate is 40-50% in Germany, 42% in the USA, 48% in Australia and 49% in France (2). No data could be obtained on the rate of use of medicinal plants and herbal products used for treatment in our country.

It has been demonstrated in different studies that extracts and essential oils of some plants growing naturally in nature show antibacterial activity against bacteria (4). While some plants with various therapeutic effects are successfully used especially in wound treatments, harmful effects may occur in others depending on the amount of use, route and form of exposure (5-6).

Extracts obtained from plants such as fixed oils, essential oils, gums, resins, etc. are used in the treatment of various diseases of both animals and humans (7). Essential oils are volatile and oil-like compounds obtained from plants or plant sources by water or water vapor distillation method (Hydrodistillation-Clevenger apparatus), which are liquid under normal conditions, sometimes freezable, with strong odor. Essential oils can be found in all organs of plants. Sometimes they can be found in all tissues of coniferous plants such as pine trees. Essential oils can be found in secretory pockets, a specific organ, secretory hairs, secretory ducts or secretory cells, varying according to the family to which the plant belongs (8).

Micromeria congesta grows naturally in the mountainous areas of Şanlıurfa, Gaziantep and Adıyaman provinces and is locally known as "Dağ nanesi", "Kaya nanesi", "Gihaye paluk", "Punge tehta", "Punk". The plant itself, its roots or above-ground parts are reported to be boiled or made into tea and drunk by the local people. It has been reported that it is used in respiratory tract disorders and cough treatment, its extract and essential oil have antimicrobial and antioxidant properties, and it is used in traditional folk medicine as antiseptic, antirheumatic, CNS stimulant and painkiller in toothache (9-10).

Rosmarinus officinalis L. is an important medicinal and aromatic plant growing naturally in Türkiye. It is also known by different names such as "kuşdili", "pürem", "hasalban", "urum çiçeği" and "akpüren" (11). In the ancient Greek and Roman periods, rosemary was used both for therapeutic purposes as a medicinal plant and for flavoring dishes in the kitchen. It has been reported that it was used especially to strengthen concentration and memory in ancient Greek times, and it was also included in some folk medicines due to its mild stimulant effect. In addition, rosemary

plant was used by burning in the environment during the Second World War in the prevention of infectious diseases, in the treatment of various diseases and in cleaning the air of patient rooms (12). Ottoman physicians stated that rosemary tea "beautifies the complexion". For this effect, they reported that either the tea should be drunk or the cream made by crushing rosemary and mixing it with butter should be used (13).

Sideritis stricta is a member of the Lamiaceae family. The genus Sideritis is represented by more than 150 species, 46 of which are found in Türkiye, and is distributed in the Mediterranean region. *S. stricta* is locally called "mountain tea", "highland tea" or "island tea" in Anatolia. It is consumed by the people as herbal tea in gastrointestinal disorders and in the treatment of colds (14). Since ancient times, the essential oils in the tea prepared from *S. stricta* have been used as folk medicine due to their physiological effects such as stimulant, carminative, digestive, stomach pain relief and appetizing due to tannins and bitter substances (15).

Artemisia absinthium L. is a perennial shrubby plant belonging to the Asteraceae family that grows along riversides, fields, slopes and steppes. Locally known as "wormwood", "bitter wormwood", "white wormwood", "great wormwood", A. absinthium grows naturally in different parts of Anatolia. It is used by local people as antipyretic, antiseptic, antihelminthic, tonic, diuretic and in stomach disorders (11,16-17). It has been reported that if it is boiled with water and drunk on an empty stomach, it is good for diabetes, delays menstruation, relieves gas and stimulates appetite (18).

Melissa officinalis L. plant belongs to the Lamiaceae family and grows naturally in the Aegean and Marmara regions of Türkiye. The leaves of lemon balm, which is especially effective on the nervous system, are used medicinally (19). Lemon balm tea is used in treatment due to its sedative, stomachic, carminative, diaphoretic and antiseptic effects. It has also been reported to be used in some regions for the treatment of depression, migraine, asthma, heart diseases, diabetes and bronchitis (20).

The aim of this study is to investigate the antibacterial activity of essential oils of some plants that are used for therapeutic purposes among the people and whose popularity has increased especially with the Covid-19 outbreak. The plants we will use in our study are *M. congesta, R. officinalis, S. stricta, A. absinthium* and *M. officinalis*.

Materials and methods

Plants and Essential Oils

M. congesta was collected from the Tektek mountains within the borders of Şanlıurfa province, *R. officinalis* was collected from the aromatic plant field at GAPTAEM Koruklu station and *S. stricta* was collected from the mountainous areas of Alanya. These plants were collected from their natural habitat and dried in June-July 2023. *A. absinthium* and *M. officinalis* plants were obtained from herbalists in dried and ready to use plastic bags. The essential oils of the plants were obtained by hydrodistillation for 6-8 hours in August using Clevenger apparatus. Different ratios of essential oil were obtained from each of the plants in our study. The essential oils were stored in dark glass bottles at +4 °C (10, 21). Dimethyl sulfoxide (DMSO) was used for homogeneous mixing of stock solutions of essential oils. Essential oil was added into DMSO at a ratio of 1:2 (v:v) to dissolve the essential oils. The mixture of essential oil and DMSO was vortex mixed at 180 rpm for 10 minutes. The stock solutions were stored in the refrigerator at +4 ^{o}C (22).

Tested Microorganisms

The bacteria used for antibacterial activity were obtained from the strain collection of Harran University, Faculty of Veterinary Medicine, Department of Microbiology

Laboratory. *Klebsiella pneumoniae, Escherichia coli, Salmonella* Typhimurium, *Proteus vulgaris* and *Staphylococcus aureus* test strains isolated from clinical samples at various times and included in the strain collection were used in our study. *Corynebacterium pseudotuberculosis* (ATCC 19410), also included in the collection as a standard strain, was used as a test strain in the study.

In Vitro Antimicrobial Activity Test

Since no standard test has been developed to evaluate the antimicrobial activity of essential oils against microorganisms, the Clinical and Laboratory Standards Institute (CLSI) method for antimicrobial susceptibility testing was used to test essential oils (22).

Agar Disc Diffusion Method

Evaluation of the essential oils in our study in terms of antimicrobial activity was performed by disc diffusion method (22-23). Bacterial cultures maintained in Trypticase Soy Broth (TSB) with 15% glycerin at -20 ^{o}C were reconstituted according to McFarland 0.5 after revitalization. Mueller Hinton Agar (MHA) was inoculated with 100 µl. After thorough spreading, the growth medium were allowed to dry (22).

Sterile filter discs (6 mm diameter) (Himedia, India) were saturated with 10 µl of stock solutions of essential oils. The impregnated discs were allowed to rest at room temperature for 30 min. Then, all discs were placed on the agar surface with bacterial cultures at regular intervals using forceps dipped in ethanol. Petri dishes were left at room temperature for 30 minutes for oil diffusion and then incubated at 37°C for 24 hours. DMSO was used as a negative control and Streptomycin and Amoxiline impregnated 6 mm diameter prepared discs (Bioanalyse, Türkiye) were used as positive controls. After incubation, inhibition zones formed around the discs were measured in millimeters and recorded (22).

Results

After 24 hours of incubation, antibacterial activity is shown in Table 1 and Figure 1.

Table 1. Antibacterial activity of the essential oils tested

Essential Oils, Antibiotics and DMSO	Tested Bacteria and Diameters of Inhibition (mm)*					
	Klebsiella pneumoniae	*Staphylococcus aureus*	*Escherichia coli*	*Salmonella* Typhimurium	*Proteus vulgaris*	*Corynebacterium pseudotuberculosis*
Melissa officinalis	10 mm	9 mm	7 mm	8 mm	8 mm	20 mm
Micromeria congesta	9 mm	11 mm	7 mm	8 mm	8 mm	20 mm
Sideritis stricta	-	-	-	-	-	-
Rosmarinus officinalis	-	-	-	-	-	-
Artemisia absinthium	-	-	-	-	-	12 mm
Streptomycin	-	10 mm	12 mm	-	8 mm	-
Amoxicillin	-	14 mm	-	12 mm	10 mm	26 mm
DMSO	-	-	-	-	-	-

*Diameter of discs included. (< 6 mm diameter length was considered as no inhibition)

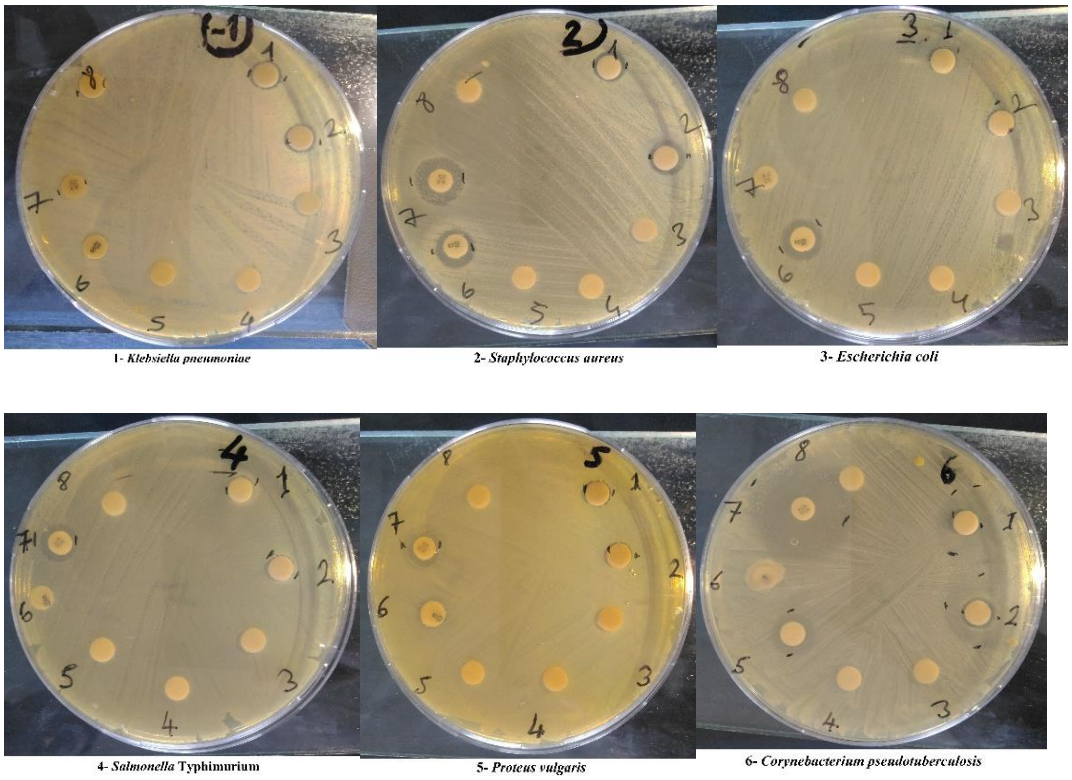

Figure 1. Antibacterial Activity Test of Essential Oils
(1. *M. officinalis* 2. *M. congesta* 3. *S. stricta* 4. *R. officinalis* 5. *A. absinthium* 6. Streptomycin 7. Amoxicillin 8. DMSO)

It was observed that the essential oils of *M. congesta* and *M. officinalis* plants tested in our study had antibacterial activity as much as reference antibiotics by disk diffusion method. It was observed that the essential oil of *A. absinthium* was effective against *C. pseudotuberculosis* bacteria, while the essential oils of other plants were not effective against the tested bacteria. As a negative control, DMSO solvent did not produce any inhibition zone against the tested strains.

Discussion

In recent years, microorganisms have developed resistance to some antibiotics used in the treatment of infectious diseases, making treatment difficult or prolonging the treatment process. The development of resistance in microorganisms may cause antibiotics to be ineffective in treatment. As a result, morbidity and mortality rates increase and cause great economic losses. The side effects of existing synthetic antibiotics and the increase in resistant bacterial species have led to increased studies on new antibacterial agents with fewer side effects (24).

In our study, it was observed that the essential oils of *M. congesta* and *M. officinalis* plants were antibacterial against the six bacterial strains tested at different levels, while the essential oil of *A. absinthium* plant was antibacterial only against *C. pseudotuberculosis* bacteria.

It has been reported that aromatic plants and essential oils of these plants have antibacterial, antiviral, antifungal, anti-inflammatory, antioxidant, antiseptic, antiparasitic, insecticidal and antitoxigenic effects and are also effective against microorganisms that have acquired resistance to antibiotics (25).

In a study, the antibacterial and antioxidant effects of *M. officinalis* essential oil were investigated. It was reported that Gram-

negative pathogenic bacteria such as *P. aeruginosa, E. coli, S. enteritidis, S.* Typhi and *Shigella* strains, especially those with multiple resistance development, showed a remarkable sensitivity to *M. officinalis* essential oil (26).

In another study in which the chemical structure and antimicrobial activity of the essential oil obtained from *M. officinalis* plant were examined, it was reported that the essential oil had different and complex chemical components. They also revealed that it had inhibition zones against all tested bacteria and showed strong antimicrobial activity with minimum inhibitor concentration (27).

In another study, the chemical composition, antioxidant potential, total phenolic content and antimicrobial activity of *M. officinalis* essential oil were investigated. It was reported that *M. officinalis* essential oil has antibacterial activity on the growth of commercial and clinical strains causing infections. They observed that gram positive bacteria were more sensitive than gram negative bacteria. They reported that bactericidal activity increased as the concentration increased (28).

In a study, the antibacterial activity of *M. congesta* essential oil and donkey milk was investigated in *E. coli* strains isolated from Urfa cheeses and developed multiple antibiotic resistance. As a result of the study, it was reported that *M. congesta* essential oil and donkey milk had higher antibacterial activity compared to reference antibiotics (tetracycline and streptomycin) (21).

In another study, the chemical composition, antimicrobial activity, total antioxidant activity, total phenol content and total oxidant status of the essential oil obtained from *M. congesta* plant were investigated. As a result of the study, it was reported that the essential oil had significant antibacterial activity and antioxidant activity compared to reference antibiotics (29).

In a study examining the chemical composition and antimicrobial activity of *A. absinthium, Artemisia scoparia* and *Artemisia sieberi* essential oils, it was reported that the essential oils of each plant had antibacterial activity against the tested bacteria depending on the concentration (30).

In another study in which the phytochemical content, antioxidant and antibacterial activities of *A. absinthium* plants collected from different regions of Tunisia were examined, it was found that the essential oil of the plant interestingly inhibited the growth of both Gram-negative and Gram-positive bacterial strains. It was reported that the essential oil of *A. absinthium* plant collected from Zaghouan region exhibited a significant inhibitory effect against *E. coli* strain with an inhibition zone of 31 mm (31).

It was observed that the antibacterial activity of the essential oils of *M. officinalis*, *M. congesta* and *A. absinthium* plants examined in our study was compatible with the literature studies. The reasons for the lack of antibacterial activity of essential oils of *S. stricta* and *R. officinalis* plants may be insufficient concentration, volatilization of essential oils in contact with air, or insensitivity to selected bacterial strains.

Conclusion

As a result, it was observed that the essential oils of *M. officinalis, M. congesta* and *A. absinthium* plants had antibacterial activity against the tested bacteria. These results show that essential oils obtained from plants can be used in the treatment of infectious diseases and traditional medicine in the future. According to the results obtained, the essential oils we examined showed different antibacterial activities. This study is important for determining the plants that grow naturally or are cultivated and have medicinal properties in our country, which has a rich plant flora. Our study will contribute to the development of

antibacterial agents in the fight against infectious diseases and will contribute to future research. More research is needed to identify the components of essential oils and reveal their effects.

Conflict of interest

The authors declare that they have no conflict of interest.

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