IJAFLS
e-ISSN: 2602-4381

International Journal of Agriculture, Forestry and Life Sciences

Original Article | Open access | Int J Agric For Life Sci (2023) 7(2): 52-55

Determining the Antimicrobial Activities of the Essential Oils of Some Taxa Used as Thyme

Neslihan Taşar[1]* **Ferit Can Yazdıc[2]** **Altuğ Karaman[3]** **Osman Gedik[4]**

[1]Munzur University, Tunceli Vocational School, Organic Agriculture Department, Tunceli, Türkiye
[2]Department of Biotechnology, Institue of Graduate Studies in Science, Munzur University, Tunceli, Türkiye
[3]Munzur University, Tunceli Vocational School, Department of Medical Documentation and Secretarial, Tunceli, Türkiye
[4]Kahramanmaraş Sütçü İmam University, Faculty of Agriculture, Department of Field Crops, Kahramanmaraş, Türkiye
***Correspondence:** ntasar@munzur.edu.tr

Citation
Taşar, N., Yazdıc. F.C., Kahraman, A., Gedik, O. (2023). Determining the Antimicrobial Activities of the Essential Oils of Some Taxa Used as Thyme. Int J Agric For Life Sci (2023) 7(2): 52-55.

Received :28 September 2023
Accepted :4 December 2023
Published Online :22 December 2023
Year: :2023
Volume :7
Issue :2 (December)
Pages :52-55

International Journal of Agriculture, Forestry and Life Sciences; Edit Publishing, Eskişehir, Türkiye.

Available online https://dergipark.org.tr/ijafls

Abstract

Many aromatic plant species that belong to the Lamiaceae family are defined as "thyme" in Turkey, and the species that contain "carvacrol" and "thymol" in the essential oil of these plants are accepted as "thyme". Among them, Origanum, Satureja, and Thymbra genera have great importance in terms of distribution and economics. The present study was conducted to determine in vitro the antimicrobial effects of the essential oils obtained from Thymbra spicata L., Satureja cuneifolia Ten., Satureja hortensis L., Origanum onites L. and Origanum majorana L. species through hydroxylation method on the pathogenic bacteria of *Salmonella enteritidis* (ATCC 13075), *Enterococcus faecalis* (ATCC 29212), *Enterobacter aerogenes* (ATCC 13048), *Staphylococcus aureus* subsp. aureus (ATCC 25923), *Escherichia coli* (ATCC 25922), and *Serratia marcescens* (ATCC 13880), which are resistant to many drugs. The essential oils that constituted the study material were extracted in the Neo-Clevenger device and their antimicrobial activity against gram-negative and gram-positive bacteria was investigated by using the Standard Agar Well Diffusion Test Method. It was found that the *T. spicata, S. cuneifolia, S. hortensis, O. onites,* and *O. majorana* species had antibacterial activity varying between 8.43±1.00-26.53±2.50 mm against six test strains. The fact that T. spicata species had higher antibacterial activity against all pathogens compared to standard antibiotics is important in terms of using and culturing this species and is the most striking finding of the present study.

Key words
Antimicrobial activity, *Thyme*, *Origanum*, *Satureja, Thymbra*.

Introduction

Approximately 3.000 (1/3) of the species that were registered in the flora of Turkey consist of medicinal and aromatic plants (Tan 2017). The Lamiaceae family, which includes the thyme plant, is among the most widespread plant families worldwide and contains 3.000 species of approximately 200 genera (Heywood 1978). It is the 6th largest family in our country with 45 genera and the 3rd largest family with 550 species in terms of species (Dönmez et al. 2011). Members of the mint family Lamiaceae are highly favored for the treatment of human and animal diseases due to their essential oil content (Güler et al., 2021; Babacan et al., 2022; Selvi et al., 2022). The fact that the Lamiaceae family is the source of many essential oils employed in medicine and perfumery and that it is employed for both treatment and spice shows the importance of this family. Thyme, which is among the most important plants of the Lamiaceae family, has five genera in Turkey (*Thymus, Origanum, Satureja, Thymbra,* and *Coridothymus*) (Baser et al. 1994, Davis 1982). There are 220 species of the genus Thymus on Earth and 39 species (58 taxa) in Turkey. There are 43 species of Origanum, of which 23 species (27 taxa) are found in Turkey. The genus Satureja contains 30 species, 13 of which (14 taxa) are found in Turkey. There are about 12 species of the genus Thymbra in Turkey, 2 species (4 taxa). There is only one species in the genus Coridothymus, which is also found in Turkey. In Turkey, 44.2% of Lamiaceae species, 65.2% of Origanum species, 52.6% of Thymus species, and 28% of Medicago species are endemic. This information indicates how abundant turkey is in these genera and that it is their genetic center (Davis 1988, Biskup and Saez 2002, Kintzios 2002).

Our country holds 70% of the world's thyme market with its rich thyme reserves, and produces approximately 10.000 tons of annual production, providing the economy of the country with an income of approximately 21 million dollars.

O. onites (Izmir thyme, earth thyme, Turkish thyme) is known in Europe as "Turkish oregano" and is widely cultivated along the Aegean and Western Mediterranean coasts (Balikesir, Izmir, Ay Den, Mugla, Antalya) (up to 1400 meters above sea level). It is cultivated in the Aegean region. This variety accounts for the largest share (about 80%) of my country's thyme exports. The average plant height can reach 100 cm. Essential oil yields range from 2% to 5%. It is thought to be the chemotype that grows in this region (Baytop 1991).

O. Majorana (sweet marjoram, Alanian thyme, marjoram, sweet thyme, white thyme) is commonly found in dry grasslands, rocks and dry forests in the western regions of our country (Thrace, Marmara, Aegean and Mediterranean). The plant is 20-80 cm tall and is a perennial plant with pink or white flowers. It blooms from July to September. It grows in the Aegean region and is used for essential oils in gardens. Its essential oil is rich in terpinen-4-ol, trans-sabinete hydrate, cis-sabinete hydrate and linalool. The carvacrol content is also quite high (78-80%). European-grown samples, known as marjoram, do not contain carvacrol (Skoula et al. 2005).

T. spicata (Karakekik, Karabaşkekik, Sivrikekik) is a common species in Thrace, the Mediterranean coast, the Aegean Sea, and western and southeastern Anatolia. Used as a medicine, with a high proportion of carvacrol for its antiseptic effect, and also in spices and teas. Carvacrol content is 50-71%. The plant height is 24-43 cm, and the essential oil proportion is between 1-3.4%.

Essential oils can be obtained from flowers, roots, bark, leaves, seeds, fruits, and various parts of plants (Khorshidian et al., 2018). Essential oils are defined as "products obtained from the peels of citrus fruits by mechanical processing, steam distillation or dry distillation after separation of the aqueous phase from natural raw materials of plant origin" (ISO/DIS9235, 2013). Today, approximately 3,000 different essential oils are used commercially as condiments and fragrances (Burt 2004). The composition of essential oils varies depending on the plant species, geographical origin, climatic conditions, soil composition, phase of the vegetation cycle, and the plant parts used to extract the essential oil (Masotti et al. 2003). In general, they play an important role in defense mechanisms against bacteria and fungi and are excreted as secondary metabolites (Tajkarimi et al. 2010). The antimicrobial activity of essential oils is related to their active components (Hyldgaard et al., 2012). Their antimicrobial properties and composition (Nychas, 1995) and their mechanisms of action have been extensively studied (Lambert et al. 2001). An important property of essential oils is their hydrophobicity, which allows them to bind to the lipids of bacterial cell membranes, disrupting their structure and making them more permeable (Sikkemat et al. 2010) and this results in the escape of ions and other cellular molecules (Gustafson 1998, Cox 2000, Carson and Riley 1993, Ultee et al. 2002). Approximately 90-95% of all essential oils consist of volatile components and fatty aldehydes, alcohols and esters as well as monoterpene and sesquiterpene hydrocarbons and their oxygenated derivatives. The remaining 5-10% of the main components are hydrocarbons, fatty acids, sterols, carotenoids, waxes, coumarins and flavonoids (Luque De Castro et al. 1999). The most active antimicrobial compounds in essential oils are divided into four classes based on their chemical structures: terpenes (e.g., paracymene, limonene), terpenoids (e.g., thymol, carvacrol), phenylpropenes (e.g., eugenol, vanillin) and other compounds such as allicin or isothiocyanates (Hyldgaard et al., 2012). The chemical structure of an essential oil also affects its antimicrobial activity (Knobloch et al., 1986). Previous research has shown that using the entire essential oil has a greater effect than using the primary ingredients together

(Burt, 2004), suggesting that secondary ingredients are critical to effectiveness and may have synergistic effects (Maruyama et al., 2005). Antagonism can be observed with essential oils, namely H. The effect of multiple compounds may be weaker than when used individually (Gill et al. 2002).

In addition to the therapeutic properties of essential oils, their antimicrobial effects have been known since the early 19th century and they have been used as disinfectants. The thyme plant, which is the subject of the present study, has a wide area of use in this context with its antimicrobial, antiseptic, anthelmintic, cardiovascular, and stimulant characteristics (Cingi et al. 1991). The purpose of this study was to compare the effects of these plants, which are important for their antimicrobial properties. The antibacterial effects of the extracted essential oils on various pathogenic bacteria were studied.

Material and methods

Supply of plant material

The localities of the plant materials that were employed to obtain the essential oils that were the subject of the study are given below.

T. spicata was obtained from natural growing areas in Kahramanmaras Menzelet Dam Lake. 37°39'2.85"K, 36°48'9.96"D, 529m.

S. cuneifolia was obtained from the natural growing area around the Hamamgozu area of Kahramanmaras Goksun District. 37°52'38.10"K, 36°23'30.59"D, 1530m.

S. hortensis Malatya, Kapidere Kumlu road surroundings. 37°56'31.94"K, 37°42'5.10"D, 1055m.

O. onites was obtained from Kahramanmaras Sutçu Imam University Field Crops Department. 37°35'37.25"K, 36°48'48.96"D, 489m.

O. majorana was obtained from Kahramanmaras Sutçu Imam University Field Crops Department. 37°35'37.25"K, 36°48'48.96"D, 489m.

Essential oil extraction

The extraction process of essential oils was carried out in the laboratory of the Department of Crops, Medicinal and Aromatic Plants, Faculty of Agriculture, Kahramanmaras Sutçu Imam University. The thyme varieties in the study samples were collected in 2018. Essential oils extracted from the above-ground parts of the plant during flowering are inspected to be pure (undiluted). The dried aerial parts of the thyme species employed in the study were completely ground and Their essential oils are extracted using hydrodistillation in a Neo-Clevenger unit for three hours. To obtain essential oil, 500 ml distilled water was added to 50 grams of ground plant material and subjected to the hydro distillation process. The resulting essential oils were stored in the refrigerator at -18^{o}C until the working setting was established.

Microorganisms employed

The bacterial species used in the study were selected from American Type Culture Collection (ATCC) quality control strains known for their sensitivity and recommended for use by the Clinical and Laboratory Standards Institute (CLSI). In this context, Enterobacter aerogenes (ATCC 13048), Enterococcus faecalis (ATCC 29212), Salmonella Enteritidis (ATCC 13075), and Staphylococcus aureus subsp. Staphylococcus aureus (ATCC 25923), Escherichia coli (ATCC 25922), and Serratia marcescens (ATCC 13880) were used in this study.

Agar well diffusion test method

The agar well diffusion test method has been used to determine the antimicrobial activity of essential oil extraction products (Russo et al., 2012) as a widely used method to evaluate the antimicrobial activity of plant or microbial extracts (Magaldi et al., 2004; Vargas et al., 2007). Muller Hinton agar (MHA) was employed for bacterial strains. Briefly, 100 µL of the pathogen sample in the logarithmic growth phase was added to 20 mL of MHA medium and poured into the Petri dish. Use a sterile cork drill (6 mm diameter) to make a hole in the MHA Petri dish and add 10 µL Necessary addition of fatty acids to each well. 10 µL of the standard antibiotic kanamycin (30 µg/mL) was also used as a positive control to determine the susceptibility of the tested microorganisms. Petri dishes were incubated overnight at 37°C, and if significant inhibition was observed, it was considered to indicate the presence of antimicrobial activity. Testing was performed in triplicate. Antimicrobial activity was assessed by measuring the diameter of the area surrounding each hole (Balouiri et al., 2016).

Statistical calculations

Statistical analysis was performed using GraphPad Prism 8 software, and all tests were performed in triplicate. Results are expressed as mean ± standard error of the mean and compared using analysis of variance (ANOVA) and least significant difference (LSD) at $p \leq 0.05$ and $p \leq 0.01$ (Steel et al., 1997).

Results

The most researched aspect of essential oils relates to their antimicrobial effects. Since these oils are complex mixtures with different components, their degree of effectiveness varies depending on the type and amount of active ingredients (Toroğlu et al., 2006). Although the information on the mechanisms of action is limited, it is suggested that this is related to the lipophilic characteristics and chemical structures of the oils (Farag et al., 1989). The essential oils that were obtained from 5 different thyme species (*T. spicata*, *S. cuneifolia*, *S. hortensis*, *O. onites*, and *O. majorana*) were tested against 6 different pathogenic microorganisms. The antibacterial activity results of the plant essential oils used in the study are shown in Table 1. The essential oils used in the study were found to exhibit broad antimicrobial activity against both gram-negative and gram-positive bacteria. *T. spicata* showed the highest antibacterial activity against all the pathogens compared to the other essential oils and standard antibiotics.

T. spicata and O. onites have strong antibacterial activity against Gram-positive bacteria, and S. cuneifolia, S. hortensis and O. onites have strong antibacterial activity against Gram-negative bacteria.

A heat map model was employed to understand the relationship between essential oils and antimicrobial activities better. In this method, the effect values of the plant species employed against bacteria are shown by the changes in blue and white. The intensity in the blue hue indicates high antibacterial activity, the white color indicates no activity, and the intermediate values indicate the corresponding gradient between the two colors (Figure 1).

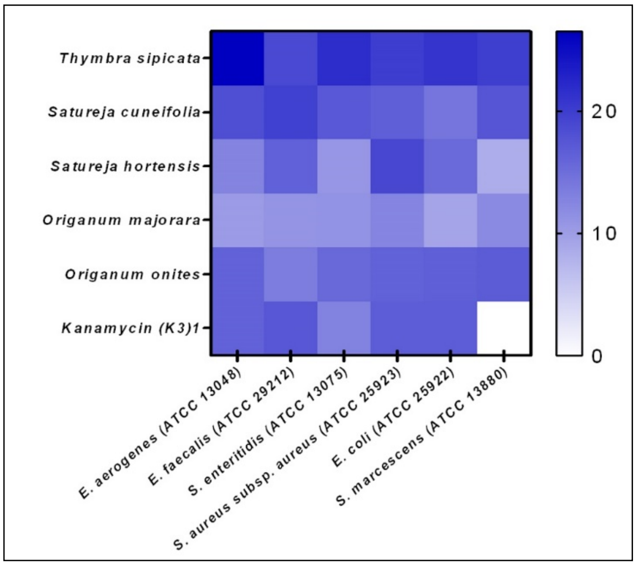

Figure 1. The comparison of antibacterial activity of essential fatty acids obtained from *T. spicata*, *S. cuneifolia*, *S. hortensis*, *O. onites*, and *O. majorana* plants and inhibition zones between pathogenic microorganisms (mm±SD).

Table 1. Data are expressed as mean ± standard deviation (SD) of three independent samples. NIZ stands for No Inhibition Zone.. 1: Kanamycin (K30), the largest zone diameter.

	Essential Fatty Acids					Standard Antibiotics
Microorganisms	***Thymbra spicata***	***Satureja cuneifolia***	***Satureja hortensis***	***Origanum majorana***	***Origanum onites***	**Kanamycin (K30)[1]**
***E. aerogenes* (ATCC 13048)**	26.53±2.50^{a}**	18.30±1.42*	12.70±1.01	10.30±0.52	16.13±0.81	16.20±0.90
***E. faecalis* (ATCC 29212)**	18.93±1.90*	19.73±1.33^{a}*	16.37±1.05	11.10±1.20	13.50±0.62	17.37±0.31
***S. enteritidis* (ATCC 13075)**	21.77±1.92^{a}**	17.23±0.35*	10.83±1.00	11.23±0.65	15.60±0.72*	12.97±0.38
***S. aureus* subsp. *aureus* (ATCC 25923)**	20.00±1.14^{a}**	16.57±0.93	19.13±0.86*	12.63±1.07	16.27±0.65	16.77±0.64
***E. coli* (ATCC 25922)**	20.97±1.63^{a}**	14.30±0.40	15.43±1.40	9.40±1.28	16.47±0.83	16.80±0.17
***S. marcescens* (ATCC 13880)**	19.97±1.87^{a}**	17.63±1.30**	8.43±1.00**	12.10±0.50**	16.90±0.26**	NIZ

*Significant effect; * P<0.05, ** P<0.01)*

Discussion and conclusion

Today, the increasing resistance of microorganisms to antibiotics is attracting people to investigate alternative drugs. Studies conducted to find plant-derived antimicrobial agents are becoming increasingly important. To this end, screening of active ingredients in plants represents an important step in the development of new antibiotic active ingredients. The antimicrobial agents to be obtained in these studies will show new horizons for the treatment of resistant microorganisms. A heat map model was prepared to demonstrate the relationship between essential oils and antimicrobial activities. The interaction between the species that formed the study sample and pathogenic bacteria is explained in this method. In this study, the diameter of the area where essential oils were ground was found to vary between 26.53 ± 2.50 and 9.40 ± 1.28. pathogen strains, were higher than those reported in previous studies and this effect was statistically significant ($P < 0.01$). These values also indicate that it has a stronger antibacterial effect than the antibiotic K30 with the largest area diameter in the control group (17.37 ± 0.31 mm). It is worth noting that even though the essential oil extracted from T. spicata had the lowest antibacterial activity (18.93±1.90), it was higher than that of the control group, showing its broad application potential.

When the positive definition of zone measurements of 7 mm and above in previous studies was considered, it was concluded that all the results obtained were high in terms of antibacterial activity (Prabuseenivasan et al., 2006; Bilenler and Gokbulut, 2019). It was found that *T. spicata*, *S. cuneifolia*, *S. hortensis*, *O. onites,* and *O. majorana* did not show antibacterial activity against gram-positive and gram-negative bacteria.

Another study examined the effects of different concentrations of thyme essential oil on various bacteria. These essential oils were found to have antimicrobial effects against Escherichia coli, Listeria monocytogenes, and Staphylococcus aureus. Studies have also found that as the concentration of essential oils decreases, so does the antibacterial effect. Studies have shown that variations in bacterial exposure can be attributed to different plant species and strains (Baydar et al., 2005).

It was also suggested that there are additives, antagonistic, and synergistic interactions between the components of essential oils (Burt 2004). In a study that examined the effects of thymol and carvacrol on *Staphylococcus aureus* and *Pseudomonas aeruginosa*, it was reported that these substances had better effects when employed together than when used alone (Lambert et al. 2001).

Akin et al. 2010) tested the essential oil obtained from *T. spicata* L. var. *spicata* through distillation method on *S. aureus* ATCC 25923, *E. faecalis* RSKK 97008, *B. cereus* ATCC 17778, E. coli ATCC 29998, *Salmonella cholerasuis* ATCC 14028, *Streptococcus mutans* NCTC 10449 and *Sarcina lutea* ATCC 9341 strains. As a result, they reported that the bacteria used were ineffective and there was no growth.

Joma (2018) tested the extracts of *T. spicata* L. var. *spicata* plant obtained with water, methanol, and hexane on *S. maltophilia* strain by disk diffusion method as 20 pl. As a result, they reported that they obtained 16 mm, 23 mm, and 10 mm zone diameters, respectively.

Also, it was reported in a previous study in which essential oils of the thyme plant were tested on 9 gram (-) and gram (+) bacteria strains that bacteriostatic effects were observed against all test microorganisms (Mahboubi et al. 2010). It was reported in some similar studies that thyme essential oil showed strong bactericidal effects on *Listeria monocytogenes* and some other bacteria (Kalemba et al. 2003).

According to the results of this study, antimicrobial activity was found in essential oils extracted from certain plants such as thyme, but this activity varied depending on the type of microorganism. The antimicrobial activity of plant essential oils varies depending on the type of microorganism tested. The results of the study show that the antibacterial effect of the essential oils used is mainly positive. Therefore, it is recognized that the use of herbal essential oils and extracts can be one of the effective solutions in this regard. The lack of mutagenicity in most plant essential oils suggests that these oils are potential sources for use as alternatives to many synthetic food additives.

Statement of Conflict of Interest

The author(s) declare no conflict of interest for this study.

Author's Contributions

NT, AK, and FCY studied antimicrobial activity. AK completed the experiments. FCY performed the statistical analysis. Plant samples were collected and identified by OG. The article was written by NT, FCY, AK, and OG. All authors edited, revised, and provided comments to the manuscript.

References

Akin, M., Demirci, B., Bagci, Y. Baser, K.H.C. (2010). Antibacterial activity and composition of the essential oils of two endemic *Salvia* sp from Turkey. *African Journal of Biotechnology*, 9, 2322-2327.

Babacan, E.Y., Polat, R., Güler, O., Moyan, A., Paksoy, M.Y. Cakilcioglu, U. (2022). An ethno veterinary study on plants used for the treatment of livestock diseases in Genc (Bingöl-Turkey). *Indian Journal of Traditional Knowledge*, 21, 81-88.

Balouiri, M., Sadiki, M. Ibnsouda, S.K. (2016). Methods for in vitro evaluating antimicrobial activity: A review. *Journal of pharmaceutical analysis*, 6, 71-79.

Baser, K.H.C., Ozek, T., Kürkcüoglu, M. Tümen, G. (1994). The Essential Oil of *Origanum vulgare* subsp. *hirtum* of Turkish Origin, *Journal of Essential Oil Research*, 6, 31-36.

Baydar, H. (2005). The Effects of Different Harvest Dates on Essential Oil Content Essential Oil Composition in *Origanum minutiflorum* O. Schwarz et. P.H.Davis. *Akdeniz University Faculty Of Agriculture Journal*, 18, 175-178.

Baytop, A. (1991). Wild and grown aromatic plants used in Turkey. *J. of Pharmacy*, 1, 76-78.

Bilenler, T. Gökbulut, I. (2019). Determination of Sensitivity of Hospital Pathogens Against Commercial Essential Oil. *Gumushane University Journal of Science Institute*, 9, 716-723.

Biskup, S. Saez, E. (2002). Thyme, The Genus *Thymus*. *Taylor-Francis*. London.

Burt, S. (2004). Essential oils: Their antibacterial properties and potential applications in foods *review. Int J Food Microbiol*, 94, 223-253.

Carson, C.F., Hammer, K.A. Riley, T.V. (2006). Melaleuca alternifolia (Tea tree) oil: A review of antimicrobial and other medicinal properties. *Clin Microbiol Rev.*, 19, 50-62.

Cingi, M.I., Kirimer, N., Sarikardasoglu, I., Cingi, C. Baser, K.H.C. (1991) Pharmacological Effects of *Origanum onites* and *O. minutiflorum* Essential Oil, *IX Herbal Medicine Raw Materials Meeting, Proceedings*, Eskisehir.

Cox, S.D., Mann, C.M., Markham, J.L., Bell, H.C., Gustafson, J.E., Warmington, J.R. Wyllie, S.G. (2000). The mode of antimicrobial action of the essential oil of *Melaleuca alternifolia* (tea tree oil). *Journal of applied microbiology*, 88, 170-175.

Davis, P. H. (1982). Flora of Turkey and The East Aegean Islands. Univ. Press, Edinburgh. Vol. 7, 36-42, 297-313.

Davis, P.H. (1988). Flora of Turkey. *Uni.Press.Edinburg*. Vol. 10.

Dönmez, M., Kargıoğlu, M. Temel, M. (2011). The Morphological, Anatomical and Ecological Properties of *Stachys palustris* L. Afyon Kocatepe, *University Journal of Science*, 11, 1-9.

Farag, R.S., Daw, Z.Y. Abo-Raya, S.H. (1989). Influence of some spice essential oils on *Aspergillus parasiticus* growth and production of aflatoxins in a synthetic medium. *J. Food Sci.*, 54, 74-76.

Gill, A.O., Delaquis, P., Russo, P. Holley, R.A. (2002). Evaluation of antilisterial action of cilantro oil on vacuum packed ham. *Int. J. Food Microbiol.*, 73, 83-92.

Güler, O., Polat, R., Karaköse, M., Çakılcıoğlu, U. Akbulut, S. (2021). An ethnoveterinary study on plants used for the treatment of livestock diseases in the province of Giresun (Turkey). *South African Journal of Botany*, 142, 53-62.

Gustafson, J.E. (1998). Effects of tea tree oil on *Escherichia coli*. *Lett. Appl. Microbiol*., 26, 194–198.

Heywood, V.D. (1978). Flowering Plands of The World. *Oxford Üniv. Press, London. p*. 239.

Hyldgaard, M., Mygind, T. Meyer, R.L. (2012). Essential oils in food preservation: Mode of action, synergies, and interactions with food matrix components. Frontiers in Microbiology, 3, 1-24.

ISO/DIS9235. (2013). Aromatic natural raw materials-vocabulary: *International Standard Organisation*. ISO 9235:2013. 9–10.

Joma, M.H. (2018). Investigation of the effect of *Thymbra spicata L. var. spicata* plant extracts on DNA protective activities and *Stenotrophomonas maltophilia*. Master Thesis, *Gaziantep University Institute of Science and Technology*, Gaziantep.

Kalemba, D. Kunicka, A. (2003) Antibacterial and antifungal properties of essential oils. *Current Medicinal Chemistry*, 10, 813–829.

Khorshidian, N., Yousefi, M., Khanniri, E. Mortazavian, A.M. (2018). Potential application of essential oils as antimicrobial preservatives in cheese. *Innovative Food Science & Emerging Technologies*, 45, 62-72.

Kintzios, E.S. (2002). Oregano, The Genus *Origanum* Lippia Taylor-Francis. London.

Knobloch, K., Weigand, H., Weis, N., Schwarm, H.M. Vigenschow, H. (1986). Action of terpenoids on energy metabolism. In Progress in Essential Oil Research: 16th International Symposium on Essential Oils, Brunke, E.J., Ed., *De Walter de Gruyter: Berlin, Germany*, pp. 429-445.

Lambert, R.J.W., Skandamis, P.N., Coote, P.J. Nychas, G.J.E. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *J. Appl. Microbiol.*, 91, 453–462.

Luque De Castro, M.D., Jiménez-Carmona, M.M. Fernández-Perez, V. (1999). Towards more rational techniques for the isolation of valuable essential oils from plants. TrAC, Trends in Analytical Chemistry, 18, 708–716.

Magaldi, S., Mata-Essayag, S. Hartung de Capriles, C. (2004). Well diffusion for antifungal susceptibility testing Int. J. Infect. Dis., 8, 39-45.

Mahboubi, M. Bidgoli, F.G. (2010). In vitro synergistic efficacy of combination of amphotericin B with *Myrtus communis* essential oil against clinical isolates of Candida albicans. *Phytomedicine*, 17, 771–774.

Maruyama, N., Sekimoto, N. Ishibashi, H. (2005). Suppression of neutrophil accumulation in mice by cutaneous application of Geranium. *essential oil. J. Inflamm*., 2, 1-11.

Masotti, V., Juteau, F., Bessiere, J.M. Viano, J. (2003). Seasonal and phenological variations of the essential oil from the narrow endemic species Artemisia molinieri and its biological activities. *Journal of*

Agricultural and Food Chemistry, 51, 7115–7121.
Nychas, G.J.E. (1995). New Methods of Food Preservation (Natural Antimicrobials from Plants). Ed. Gould G.W. Publisher: *Springer US Copyright: Holder Chapman & Hall, pp* 58-89.
Prabuseenivasan, S., Jayakumar, M. Ignacimuthu, S. (2006). In vitro antibacterial activity of some plant Essential oils. *Biomedical Central*, 6, 39-45.
Russo, A., Formisano, C. Rigano, D. (2013). Chemical composition and anticancer activity of essential oils of Mediterranean sage (*Salvia officinalis* L.) grown in different environmental conditions. *Food Chem Toxicol*., 55, 42–47.
Selvi, S., Polat, R., Cakılcıoglu, U., Celep, F., Tuncay Dirmenci, T. Ertug, Z.F. (2022). An ethnobotanical review on medicinal plants of the Lamiaceae family in Turkey. *Turkish J Botany*, 46, 283-332.
Sikkemat, B.J., Bontt, J.A.M. Poolmann, B. (2010). Interactions of Cyclic Hydrocarbons with Biological Membranes. *J. Biol. Chem.*, 269, 8022–8028.
Skoula, M. Grayer, R.J. (2005). Volatile Oils of *Coridothymus capitatus, Satureja, Thymbra, Satureja spinosa* and *Thymbra calostachya* (Lamiaceae) from Crete, *Flavour Fragr. J.*, 20, 573-576.
Steel, R.G.D., Torrie, J.H. Dicky, D.A. (1997). Principles and Procedures of Statistics, A Biometrical Approach. 3rd Edition, *McGraw Hill, Inc. Book Co.*, New York, 352-358.
Tajkarimi, M.M., Ibrahim, S.A. Cliver, D.O. (2010). Antimicrobial herb and spice compounds in food. *Food Control*, 21, 1199–1218.
Tan, N., Satana, D., Sen, B., Tan, E., Altan, H.B., Demirci, B. Uzun, M. (2016). Antimycobacterial and Antifungal Activities of Selected Four *Salvia* Species. *Records of Natural Products*. 10, 593-603.
Toroglu, S. Cenet, M. (2006). The Methods Used To Determine Antimicrobial Activity Of The Plants And Their Application Area. *KSU Journal of Science and Engineering*, 9, 12-20.
Ultee, A., Bennik, M.H.J. Moezelaar, R. (2002). The Phenolic Hydroxyl Group of Carvacrol Is Essential for Action against the Food-Borne Pathogen Bacillus cereus. *Applied and Environmental Microbiology, Apr.*, 68, 1561–1568.
Valgas, C., De Souza, S.M. Smânia, E.F.A. (2007). Screening methods to determine antibacterial activity of natural products Braz. *J. Microbiol.*, 38, 369-380.