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Research Article

Antimicrobial activities of some species in Asteraceae and Lamiaceae families from Türkiye

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Abstract: The study aimed to investigate the antimicrobial activities of the species belong to Asteraceae and Lamiaceae families collected from Çorum, Eskişehir and Kütahya provinces in Türkiye on Gram (+) and Gram (-) bacterial strains using EUCAST disc diffusion method. Ethanol (96%) and Ciprofloxacin (5mcg) were used as negative and positive controls, respectively. All plant extracts produced inhibition zones on *S. aureus* and *E. faecalis* ranged between 4.67-14.33 mm and 21.67-23.67 mm respectively. The variance in the antimicrobial activities of the plant extracts was significant between groups according to ANOVA. *L. angustifolia* samples collected from Eskişehir and Kütahya gave zone diameters close to the positive control on *S. aureus* and *E. faecalis*. It was determined that *E. coli* was the most resistant and *S. aureus* and *E. faecalis* were the most sensitive microorganisms in this study. *L. angustifolia*-E, *M. piperita* and *S. officinalis* were the species whose extracts were coming front with their high antimicrobial activities. *Pearson*'s correlation analyses displayed that the antimicrobial activity on *E. coli* was correlated positively and negatively with altitude and latitude respectively, while on *E. faecalis* was positively correlated with altitude and negatively correlated with latitude and longitude. In conclusion, the variations in the antimicrobial activities of the secondary metabolites found in the extracts of medicinal aromatic plants are important and, although the quantity, quality, and diversity of these compounds are determined according to the genotypes of plants, the environmental conditions in which the plants grow might have an impact on these differences.

1. INTRODUCTION

When we think about what life would be like without pathogenic microorganisms and the infectious diseases they cause? We would probably have a healthier and happier life than living with pathogens. Unfortunately, they exist and are part of life on earth, and we have to deal with them. No matter how much progress has been made in medicine and pharmacology in developing new drugs, especially antibiotics, antibiotic resistance remains as an important problem. In addition, it is estimated that by 2050, the number of people affected and dying from infections caused by pathogenic microorganisms that are resistant to existing antibiotics will

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exceed 10 million (Goff *et al*., 2017; Heidarian *et al*., 2022). Therefore, in order to develop alternative treatment methods to antibiotics, the use of secondary metabolites obtained from medicinal and aromatic plants has become one of the remarkable issues recently. Secondary metabolites are used not only as alternative methods in the treatment of diseases, but also in the cosmetic industry, aroma therapies, food packaging for extending shelf life, and surface disinfection products due to their antimicrobial activities.

Türkiye is one of the countries with a rich diversity in terms of medicinal aromatic plant species. Many researchers in the world and in Türkiye are conducting research on various activities of these plants such as antimicrobial, antifungal, antiviral, or antitumor. While previous studies state that the compounds that make up the chemical content of aromatic plants are controlled by their genotypes, they indicate that the environment in which they grow might also have an impact on the variations in the compounds extracted by different extraction methods from the plants growing in different environments (Burt, 2004; Saharkhiz *et al*., 2009; Andry *et al*., 2017). Increasing the number of these studies will make significant contributions to both the economy and healthy life in order to benefit from these plants more efficiently. Synthetically produced medicines also originate from the compounds found in plants in nature. Therefore, the investigation and determination of the antimicrobial activities of different medicinal aromatic plant species in this study will provide new information to the literature for more comprehensive new studies to be conducted with these plant species or other species in the future.

Some species from the Asteraceae and Lamiaceae families used in this study and previous studies:

Species belonging to the Asteraceae family: The family includes genera and species that are distributed worldwide and have economically important medicinal and aromatic properties.

Echinacea purpurea L Moench: *E. purpurea* is a herbaceous perennial with various pharmacological properties and a medicinal plant with purple flowers. It is known that the *Echinacea* genus originated in the United States and later spread to Europe (Hudson, 2012). Today, it is grown in many places in the world. There are nine different known species of *Echinacea*, and three of them (*Echinacea purpurea*, *Echinacea pallida* (Nutt.) Nutt. and *Echinacea angustifolia* DC.) are used as medicinal plants (Burlou-Nagy *et al*., 2022). It has been revealed in previous studies that the essential oils of *E. purpurea* show antimicrobial, antioxidant, anticancer and antifungal activities (Algabar *et al*., 2022).

Achillea filipendulina Lam.: *A. filipendulina* (yarrow) is an Asian species of the Asteraceae family, native to Central and Southwest Asia (Afghanistan, Iraq, Pakistan, Iran, Türkiye, Kazakhstan, and the Caucasus) (Asnaashari, 2023). *A. filipendulina*, an aromatic plant with bright yellow flowers, blooms from June to September. The extract of this plant is used to treat various diseases such as arthritis, gastrointestinal problems, blockages, and malaria. Little work has been done on the essential oils of species belonging to the *Achillea* genus (Kaur *et al*., 2017). It has been reported that the aerial parts of the *Achillea* genus and the different species it contains have properties such as antioxidant, anti-inflammatory and antimicrobial activities (Kaur *et al*., 2017).

Species belonging to the Lamiaceae *family*: Lamiaceae is one of the most widespread plant families containing a wide variety of species from an ethnomedical perspective. The Lamiaceae family contains approximately 250 genera and 6,900 to 7,200 species (Napoli *et al*., 2020).

Salvia officinalis: *Salvia* containing 900 species is the largest genus in the Lamiaceae family. *S. officinalis*, known as garden sage, is a plant native to the Mediterranean region. Garden sage is a perennial evergreen shrub with woody stems, grayish leaves, and blue to purplish flowers (Ahl *et al*., 2015). While garden sage is widely consumed as herbal tea among the people, its essential oils are widely used in the pharmaceutical and cosmetic industries. According to previous studies, garden sage essential oils have antimicrobial activity (Abu Darwish, 2014; Sulaiman *et al*., 2023) and are used therapeutically in diseases such as antirheumatic, diuretic, expectorant, insecticide, laxative (monoterpenes), analgesic, antiarrhythmic, antiepileptic and spasmolytic (Velickovic *et al*., 2003).

Melissa officinalis L.: Lemon balm (*M. officinalis*) is a medicinal plant with high antimicrobial activity and is distributed in the Middle East, Central Asia and some parts of Europe. Previous phytochemical studies showed that this plant contains volatile compounds, triterpenoids, phenolic acids and flavonoids (Shakeri, 2016), and these metabolites have antibacterial (Hassan *et al*., 2019), antifungal (Abdel Naime *et al*., 2019; Heidarian *et al*., 2022) activities. *M. officinalis* as a medicinal plant has long been used in different ethno-medical systems in the treatment of various diseases and disorders such as headaches, irritability, colic, indigestion, heart failure, depression, and rheumatism in traditional medicine (Shakeri 2016).

Mentha piperita L.: Although peppermint (*M. piperita*) is a native species of the Mediterranean region, a medicinal aromatic plant grows in many parts of the world. Its leaves and flowers have medicinal properties. The most common compounds found in peppermint oil are menthol and menthone (Derwish *et al*., 2010). These compounds have made peppermint oil a valuable natural product preferred as a raw material in many fields such as aromatherapy and phytotherapy in the cosmetic industry. Apart from these, peppermint is a plant that is in demand as seasoning, in medicine, pharmacy, and food industry due to its smell and flavour. Additionally, according to previous studies, it has also been described that peppermint essential oil has antibacterial (Kızıl *et al*., 2010; Gruľováa 2016; Afridi *et al*., 2016) and antifungal (Kızıl *et al*., 2010; Saharkhiz *et al*., 2012) activities.

Lavandula: It is a member of the family Lamiaceae and belongs to the subfamily Nepetoideae. A number of tribes are recognized within the Nepetoideae, and Lavandula is currently treated as its own separate and isolated group, namely the *Lavanduleae* (Endl.) Boiss tribe, which contains only a single genus *Lavandula* (Lis-Balchin, 2002). The genus *Lavandula* contains approximately 39 species (Upson and Andrews, 2004). It is widespread in the Mediterranean region (Soheili and Salami, 2019), in the Canary Islands and India (Upson and Andrews, 2004), in northern, eastern and southern Africa, Bulgaria, Spain, Poland, Türkiye, France, England, Russia, Australia and the USA (Śmigielski *et al*., 2009).

Lavandula angustifolia (lavender) and *Lavandula* × *intermedia* (Emeric ex Loisel.) (lavandin): Lavender and lavandin essential oils contain various secondary metabolites such as coumarins and phenolic compounds (Panuccio *et al*., 2016). In previous studies, it has been reported that the essential oils isolated from these plants were found to have antimicrobial (Nikšić *et al*., 2017), antifungal (Slimani *et al*., 2022), antioxidant (Andrys *et al*., 2017) and herbicide (Xiaotian *et al*., 2020) activities.

The aims of this study are

i. to investigate antimicrobial activities of medicinal aromatic plants [*M. piperita* (Peppermint), *S. officinalis* (Garden sage), *M. officinalis* (Lemon balm), *A. filipendulina* (Yarrow), *E. purpurea* (Echinacea), *L. angustifolia* (lavender) and *L.* × *intermedia* (Lavandin)] on some Gram (+) [*E. faecalis* ATCC 29212, *S. aureus* ATCC 29213] and Gram (-) [*E. coli* ATCC 25922, *P. auriginosa* ATCC 27853] bacterial strains, and whether they differ in their antimicrobial activities,
ii. to find whether the antimicrobial activities of plant ethanol extracts differ according to bacterial species,
iii. to find whether plant ethanol extracts applied at three different doses (15, 20 and 25 μL) differ in their antimicrobial activities,

iv. to find whether there is a correlation between antimicrobial activities and the geological data (altitude, latitude and longitude) of the locations where the plant samples were collected, and the ethanol extracts applied at different doses.

2. MATERIALS and METHODS

1.1. Materials

In this study, the dried flowers and leaves of five different aromatic plant species [*M. piperita*, *S. officinalis*, *M. officinalis*, *A. filipendulina* and *E. purpurea*], obtained from a medicinal aromatic plant breeder (HMC Naturel) in Çorum Dodurga, and two lavandula species [*L. angustifolia* and *L.* × *intermedia*] grown by farmers, collected from Eskişehir, Çifteler, Belpınar Village, Kütahya, Merkez, Aloğlu Village, Çorum, Alaca, Gerdekkaya Village were used. Detailed information about the plants is given in Table 1. In the investigation of the antimicrobial activities of plant ethanol extracts, Gram (+) [*E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213] and Gram (-) [*E. coli* ATCC 25922 and *P. auriginosa* ATCC 27853] bacterial strains were used, while ethanol (96%) and Ciprofloxacin (5mcg) antibiotic disc were used as negative and positive controls respectively.

Table 1. Detailed information about the plant material used in the ethanol extraction process (Abbreviations: A: altitude, LA: latitude, LO: longitude).

	Species Name	Local Name	Location Name	A (m)	LA	LO
1.	*Mentha piperita* L.	Tıbbi nane	Dodurga/Çorum	607	40.51	34.48
2.	*Salvia officinalis* L.	Adaçayı	Dodurga/Çorum	607	40.51	34.48
3.	*Melissa officinalis* L.	Melisa	Dodurga/Çorum	607	40.51	34.48
4.	*Achillea filipendulina* Lam.	Civanperçemi	Dodurga / Çorum	607	40.51	34.48
5.	*Echinacea purpurea* L.	Ekinezya	Dodurga / Çorum	607	40.51	34.48
6.	*Lavandula angustifolia* Mill.	Lavanta	Eskişehir, Çifteler, Belpınar Village	936	39.31	31.67
7.	*Lavandula* × *intermedia* Emeric ex Loisel.	Lavanta	Çorum, Alaca, Gerdekkaya Village	1100	40.34	35.35
8.	*Lavandula angustifolia* Mill.	Lavanta	Kütahya Merkez, Aloğlu Village	1247	39.35	29.94

1.2. Methods

1.2.1. *Extraction with Soxhlet device*

The plant materials were dried in an environment protected from sunlight, and the flower (lavandula, Echinacea, yarrow) and leaf (peppermint, garden sage, and lemon balm) parts of the plants were ground in a porcelain mortar at room temperature. The grounded plant powder was filled in a filter bag, placed in the cartridge chamber of the Soxhlet device and the extracts were obtained by using 300 mL of ethanol (96%) as the solvent and performing the extraction process for 24 hours at a temperature not exceeding the boiling point of the solvent. After the extraction finished, the extraction solution containing the extracted solutes was left to evaporate until approximately 30 mL, which was transferred to the fresh tubes (50 mL) and they were centrifuged at 2000 rpm for 20 min. The supernatant was transferred to fresh amber glass vials, evaporated until solid extracts remained, and kept at + 4°C until use. After the soluble extracts were dissolved with the same solvent (5 mL) used for extraction, the solutions were filtered through 0.22 µm pore size micro filters into capped fresh amber glass bottles and stored at +4 °C until used for antimicrobial analyses.

1.2.2. *Bacterial strains and growth media*

In the study, Gram (+) (*E. faecalis* ATCC 29,212 and *S. aureus* ATCC 29,213) and Gram (-) (*P. aeruginosa* ATCC 2753 and *E. coli* ATCC 25,922) in total four different bacterial strains were used for antimicrobial analyses. Bacterial strains were grown in different growth media in the following order; blood agar, broth, and nutrient agar at their respective optimum growth temperatures (37°C). Luria-Bertani (LB) was used to store the bacterial strains at – 20 ºC. Bacterial suspensions were adjusted to a 0.5 McFarland standard, which is equivalent to a bacterial suspension containing 1 × 10^8 to 2 × 10^8 CFU/mL *E. coli* using sodium chloride physiological solution.

1.2.3. *Disc diffusion method*

The determination of antimicrobial activity was performed according to the EUCAST disc diffusion method (www.eucast.org, 2023). The same petri dishes were used to observe the antimicrobial activities of the plant extracts on the microorganisms and to observe the control groups. The bacterial stains were inoculated on the Müller Hinton agar medium by spreading method to determine the antimicrobial activities of the plant extracts and then, the sterile standard discs were impregnated at equal distances on the medium using a sterile forceps by pressing lightly. Application of three different doses of 15, 20, and 25 μL of plant extracts was carried out in three replicates. After the discs were placed for 15 minutes, the petri dishes were incubated in an incubator at 37 ºC for 24 hours. Commercially available standard Ciprofloxacin antibiotic disc (5mcg) was used for positive control, while ethanol (96%) was used as negative control. The evaluation was made by measuring the diameters of the inhibition zones around the discs, where the bacterial strains did not grow, in mm with a ruler. The experiments were performed in three replicates, the scores of the results were recorded for each trial, and then, the mean values of the inhibition zone diameters were used for the statistical analysis.

1.2.4. *Statistical analyses*

The mean values obtained by evaluating the antimicrobial analyses were used to determine the significance of the variance in the antimicrobial activity of the plant extracts.

One-way ANOVA: The test of Homogeneity of Variances (THV) test was applied to determine the homogeneity of sample distributions in the groups. According to the results of THV, it was determined that the sample distribution in the groups was equal, and the Sigma (*p*) value was greater than 0.05 in some groups and lower than 0.05 in some groups. Whether the variances observed in the antimicrobial activities of plant extracts on the bacterial strains were significant within and between groups was evaluated with one-way ANOVA test. Since the sample distribution was equal and homogeneous in all groups, Tukey's HSD and Sheffe *post hoc* tests (data not given) were applied along with the ANOVA.

Pearson's correlations: *Pearson*'s correlation coefficient was calculated to determine whether there was a correlation between the mean values of the antimicrobial inhibition zone diameters obtained as a result of the application of the plant extracts, ethanol (96%), and the Ciprofloxacin (5mcg) and the type of bacteria, the application of plant extracts in different volumes (15, 20, and 25 μL), and the geological data (altitude, latitude and longitude) of the locations, where the plant samples were collected. Correlation analyses were performed with IBM SPSS (ver. 22).

3. RESULTS

1.3. Antimicrobial Activities

Ethanol extracts obtained from the plants used in the analyses were applied in three replicates on four different bacterial strains in three different volumes. Ethanol was used as negative control and Ciprofloxacin antibiotic (5mcg) disc was used as positive control and standard. The mean values of the inhibition zone diameters were calculated and given in Table 2. No antimicrobial activity was observed in all negative control applications.

Table 2. The mean values of the inhibition zone diameters resulting from the antimicrobial activities of the plant extracts, the positive and negative controls.

	Species Name	*Escherichia coli* ATCC 25922			*Pseudomonas auriginosa* ATCC 27853			*Staphylococcus aureus* ATCC 29213			*Enterococcus faecalis* ATCC 29212		
		15 µL	20 µL	25 µL	15 µL	20 µL	25 µL	15 µL	20 µL	25 µL	15 µL	20 µL	25 µL
1	*Mentha piperita*	0	0	2.67	2.33	7.00	8.00	12.00	14.33	14.33	6.33	9.33	11.33
2	*Salvia officinalis*	0	0	0	2.33	2.33	7.00	8.67	14.33	14.00	10.33	11.33	12.00
3	*Melissa officinalis*	0	0	0	0	2.33	7.33	6.33	9.67	11.33	7.67	5.33	7.67
4	*Achillea filipendulina*	0	0	0	0	0	4.67	9.67	6.33	11.33	0	2.67	8.00
5	*Echinacea purpurea*	0	0	0	0	0	4.67	5.67	5.67	4.67	3.00	2.33	7.67
6	*Lavandula angustifolia*-E	8.33	8.00	5.67	4.67	0	8	8.67	10.67	12	14.33	22	20.67
7	*Lavandula intermedia*- Ç	7.00	8.00	5.33	0	0	4.67	8.00	9.67	9.33	7.67	15.67	11.00
8	*Lavandula angustifolia*-K	2.33	8.00	5.67	0	0	0	7.67	10.33	11.33	9.67	20.33	18.00
N.C	Ethanol	0	0	0	0	0	0	0	0	0	0	0	0
P.C	Ciprofloxacin (5mcg)	34.00	35.00	33.67	31.67	31.33	32.00	29.00	30.00	30.00	21.67	22.00	23.67

NC: Negative control, PC: Positive control, E: Eskişehir, Çifteler, Belpınar Village, Ç: Çorum, Alaca, Gerdekkaya Village, K: Kütahya, Merkez, Aloğlu Village.

Antimicrobial activities on E. coli ATCC 25922: It was observed that the ethanol extracts obtained from *M. piperita*, *S. officinalis*, *M. officinalis*, *A. filipendulina* and *E. purpurea* plants did not have any antimicrobial activities on *E. coli* (Figure 1a). On the other hand, it was observed that the ethanol extracts of the *Lavandula* species collected from three different locations had antimicrobial activities (8.33-2.33 mm) on *E. coli*, but they showed approximately a quarter of the activity compared to the positive control (33.67-35 mm).

Antimicrobial activities on P. auriginosa ATCC 27853: When the plant ethanol extracts were applied in 15 µL and 20 µL volumes, it was observed that the *A. filipendulina*, *E. purpurea*, and *L. intermedia*-Ç samples did not show any antimicrobial activities, while the *L. angustifolia*-K did not show antimicrobial activity in all three volumes applied (Figure 1b). The inhibition zone diameters produced by *M. piperita* and *S. officinalis* in 15 µL, 20 µL and 25 µL, *M. officinalis* in 20 µL and 25 µL, *A. filipendulina* and *E. purpurea* in 25 µL, *L. angustifolia*-E in 15 µL and 25 µL ranged between 2.33-8.00 mm, 2.33-7.33 mm, 4.67 mm, and 4.67-8.00 mm respectively. It was determined that the positive control showed antimicrobial activity at approximately four times larger than the plant extracts.

Antimicrobial activities on S. aureus ATCC 29213: All the plant extracts applied in three volumes showed antimicrobial activities on *S. aureus* at rates ranging from 4.67 to 14.33 mm (Figure 1c) while inhibition zone diameters were observed in the range of 29-30 mm by the positive control. When the plant extracts were applied in 20 µL and 25 µL volumes, it was determined that *M. piperita* and *S. officinalis* showed higher activity than the other plant extracts.

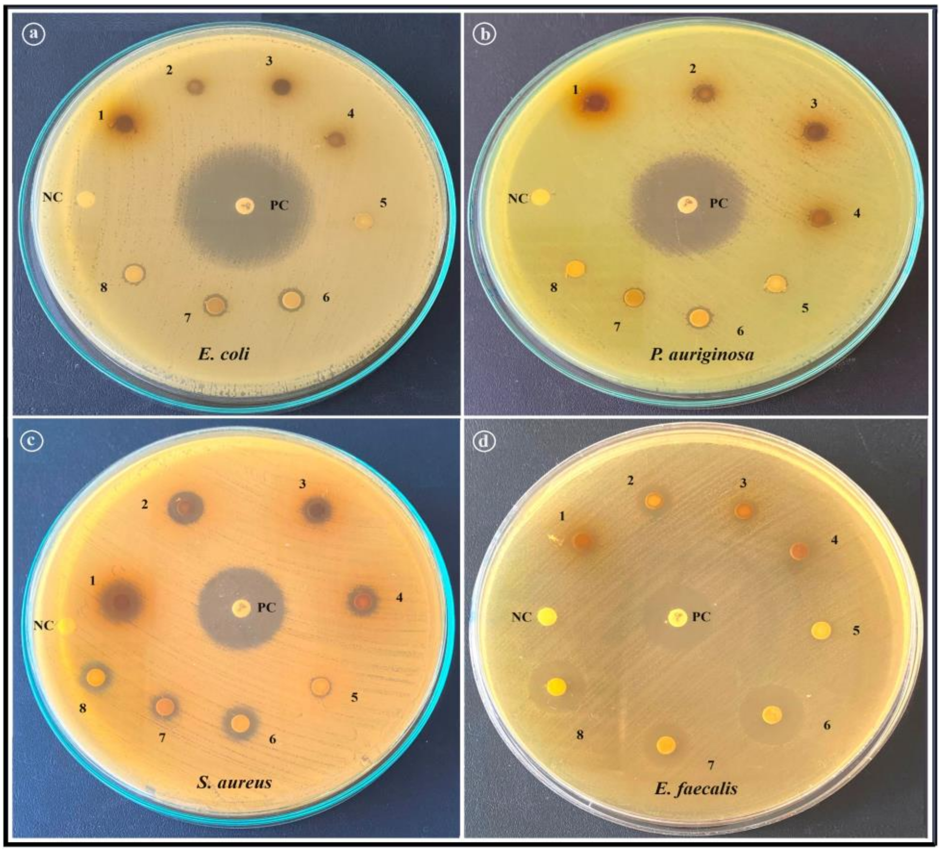

Figure 1. The inhibition zones formed by the antimicrobial activities of the aromatic plant species' extracts applied in 25 µL volumes together with positive and negative controls on the bacterial strains; a) *E. coli*, b) *P. auriginosa*, c) *S. aureus*, and d) *E. faecalis* (The numbers on the figures are representing the plant species in the same order given in Table 1, NC: Negative control, PC: Positive control)

Antimicrobial activities on E. faecalis ATCC 29212: It was determined that all plant extracts showed antimicrobial activities ranging from 2.67-20.67 mm in all three applied volumes on *E. faecalis* (Figure 1d), the positive control showed the inhibition zone diameters ranging from 21.67 to 23.67 mm. The significant point in this application is that *L. angustifolia*-E plant extract showed inhibition zone diameters ranging from 14.33 to 22 mm very close to the positive control.

1.4. Analysis of Variance According to Four Different Bacterial Groups

One-way ANOVA was applied to determine whether there was a statistically significant difference in the antimicrobial activities of the eight different plant extracts analyzed according to four different bacterial strains. According to the ANOVA results, it was revealed that the antimicrobial activities of all plant extracts and positive control showed significant differences between the groups based on *Sigma* values. ANOVA values were calculated in the range of $F = 4.52$ ($p = 0.04$ significant at the 0.05 level) to $F = 148$ ($p = 0.00$ significant at the 0.05 level) (Table 3).

Table 3. According to ANOVA, the significance of the variances of the antimicrobial activities of eight aromatic plants' ethanol extracts and positive control on the bacterial strains.

Species Name		Sum of Squares	*df*	Mean Square	*F*	Sig.
Mentha piperita	BG	256.17	3	85.39	17.36	0.00
	WG	39.36	8	4.92		
	Total	295.53	11			
Salvia officinalis	BG	314.60	3	104.87	23.22	0.00
	WG	36.14	8	4.52		
	Total	350.74	11			
Melissa officinalis	BG	145.44	3	48.48	8.68	0.01
	WG	44.67	8	5.58		
	Total	190.11	11			
Achillea filipendulina	BG	142.47	3	47.49	6.26	0.02
	WG	60.69	8	7.59		
	Total	203.16	11			
Echineceae. purpurea	BG	54.51	3	18.17	4.52	0.04
	WG	32.13	8	4.02		
	Total	86.64	11			
Lavandula angustifolia-E	BG	364.29	3	121.43	12.83	0.00
	WG	75.72	8	9.47		
	Total	440.01	11			
Lavandula intermedia-Ç	BG	159.90	3	53.30	8.19	0.01
	WG	52.04	8	6.50		
	Total	211.94	11			
Lavandula angustifolia-K	BG	414.21	3	138.07	12.81	0.00
	WG	86.22	8	10.78		
	Total	500.43	11			
Ethanol (N.C)	BG	0.00	3	0.00	-	-
	WG	0.00	8	0.00		
	Total	0.00	11			
Ciprofloxacin (5mcg)	BG	230.34	3	76.78	148.02	0.00
	WG	4.15	8	0.52		
	Total	234.49	11			

*Sigma $p < 0.05$ is significant.
Abbreviations: E: Eskişehir, Çifteler, Belpınar Village, Ç: Çorum, Alaca, Gerdekkaya Village, K: Kütahya Merkez Aloğlu Village.

Post hoc tests: When it was determined that the variance between groups was significant, Tukey's HSD and Scheffe were performed as *post hoc* tests to determine the origin of differences. According to the results of *post hoc* tests, when the groups were compared with each other, *Sigma* values showed that the antimicrobial activities of the aromatic plant extracts and positive control applied on four different bacterial strains differed between bacterial groups and this difference was significant (data not given). The least difference between bacterial groups was found after the application of *E. purpurea* extract.

1.5. *Pearson*'s Correlation

Whether there was a correlation between the antimicrobial activities of aromatic plant ethanol extracts on four bacterial strains and the geographical conditions (altitude, latitude, and longitude) of the locations, where the plant samples were collected was analyzed by calculating *Pearson's* correlation coefficients. According to the results, when the aromatic plant extracts were applied in 20 µL and 25 µL volumes, there were positive correlations between the antimicrobial activities on *E. coli* and altitude (r_P = 0.950, p = 0.000, significant at the 0.01 level and r_P = 0.896, p = 0.003, significant at the 0.05 level, respectively) (Table 4).

Table 4. *Pearson's* correlations between the antimicrobial activities of the aromatic plant extracts and the geographical conditions of the locations, where the plant samples were collected.

Bacterial strains	V	r_P	A (m)	LA	LO
Escherichia coli ATCC 25922	15 µL	r_P	0.688	-0.635	-0.301
		p	0.059	0.091	0.469
	20 µL	r_P	0.950**	-0.814*	-0.604
		p	0.000	0.014	0.113
	25 µL	r_P	0.896*	-0.790*	-0.599
		p	0.003	0.020	0.117
Pseudomonas auriginosa ATCC 27853	15 µL	r_P	-0.051	-0.401	-0.243
		p	0.904	0.325	0.561
	20 µL	r_P	-0.463	0.397	0.294
		p	0.248	0.330	0.479
	25 µL	r_P	-0.635	0.360	0.503
		p	0.091	0.381	0.204
Staphylococcus aureus ATCC 29213	15 µL	r_P	-0.131	0.058	0.070
		p	0.757	0.892	0.869
	20 µL	r_P	0.015	-0.068	-0.070
		p	0.973	0.872	0.870
	25 µL	r_P	-0.061	-0.105	-0.140
		p	0.887	0.804	0.741
Enterococcus faecalis ATCC 29212	15 µL	r_P	0.474	-0.665	-0.518
		p	0.235	0.072	0.189
	20 µL	r_P	0.824*	-0.853**	-0.690
		p	0.012	0.007	0.058
	25 µL	r_P	0.678	-0.934**	-0.826*
		p	0.065	0.001	0.011
	N		8	8	8

**Correlation is significant at the 0.01 level (2-tailed), *Correlation is significant at the 0.05 level (2-tailed).
Abbreviations: V: Volume, A: altitude, LA: latitude, LO: longitude, N: Sample size, r_P: Pearson's correlation coefficient, p: p-value.

It was determined that there were negative correlations (r_P = -0.814, p = 0.014, significant at the 0.05 level and r_P = -0.790, p = 0.020, significant at the 0.05 level, respectively) between antimicrobial activities when 20 µL and 25 µL volumes applied on *E. coli* and latitude. When the plant extracts were applied in a volume of 20 µL, the observed antimicrobial activity on *E. faecalis* was positively correlated with altitude (r_P = 0.824, p = 0.012, significant at the 0.05 level) and negatively correlated with latitude (r_P = -0.853, p = 0.007, significant at the 0.05 level). When it was applied in 25 µL volume on *E. faecalis*, there were negative correlations between antimicrobial activities and both latitude and longitude (r_P = -0.934, p = 0.001, significant at the 0.01 level and r_P = - 0.826 p = 0.011, significant at the 0.05 level, respectively).

4. DISCUSSION and CONCLUSION

The antimicrobial activities of the plant extracts obtained with ethanol on four different bacterial strains were investigated in the study. It was observed that there were variations in the antimicrobial activities of extracts from different aromatic plant species analyzed. When the mean values of inhibition zone diameters were evaluated, it was determined that plant extracts varied in terms of their antimicrobial activities on different bacterial strains. When these results were evaluated in general, it was determined that extracts of the aromatic plant species other than Lavandula species had no activity on *E. coli*, but Lavandula species showed activity, albeit lower, than the positive control. According to these results, it was found that *E. coli* was the most resistant microorganism against the antimicrobial activities of plant extracts used among the bacterial strains analyzed. Since the analyzes on *P. auriginosa* showed antimicrobial activity, albeit at low rates, depending on the application of the plant extracts in different volumes, *P. auriginosa* can be defined as the most resistant species in the second order. It was revealed that plant extracts showed intense antimicrobial activities on *S. aureus* and *E. faecalis* compared to others. When antimicrobial activities are compared, it was clearly seen that the plant extracts have higher effects on *S. aureus* than on *E. faecalis*. *A. filipendulina* and *E. purpurea* plant extracts displayed lower antimicrobial activities on the bacterial strains analyzed than the other plant species, while *Lavandula* species showed the highest activity. In addition, it was observed that the activity of *Lavandula* species on *E. faecalis* was almost the same as the positive control. These results indicate that medicinal aromatic plants potentially contain naturally occurring metabolites with high antimicrobial activity. The plant species, whose antimicrobial activities were analyzed in this study were determined to show similar activities especially on the same or different microorganisms in previous studies such as *M. piperita*, (Gruľová *et al*. 2016; Afridi *et al*. 2016), *S. officinalis* (Sulaiman *et al*. 2023), *M. officinalis* (Hassan *et al*., 2019; Heidarian *et al*., 2022), *A. filipendulina* (Kaur *et al*., 2017), *E. purpurea* (Rubinstein *et al*., 2008), *Lavandula genus* (*L. angustifolia*, and *L.* x *intermedia*) (Andrys *et al*. 2017; Nikšić *et al*. 2017).

When the contents of plant essential oils are analyzed using methods such as GC-MS, it is known that they are a mixture of many bioactive compounds. When their biological activities are investigated, it is generally unknown whether or how they interact with each other synergistically, since they are all administered together in the extract. The compositions of these essential oils may vary according to species, seasons, between populations (Vokou, 1993), and even individuals within the population (Tarayre *et al*. 1995; Gruľová *et al*. 2016).

It has also been shown in clinical studies that *M. officinalis* contains triterpenoids, phenolic acids, and flavonoids in its chemical composition and has effects on mood, cognition, and memory (Shakeri *et al*., 2016). Additionally, the compounds responsible for antibacterial and antifungal activities have been reported to be citrals (geranial and neral) and citronennal (Mimica-Dukic *et al*. 2004). Although not statistically significant, *M. officinalis* has also been reported to increase ocular pressure and inhibit thyroid hormone as side effects (Shakeri *et al*., 2016).

There are various compounds in the components of peppermint (*M. piperita*) essential oil. Gruľová *et al*. (2015) found that the components of peppermint oil were (-)-menthol (58.7-71.2%), menthone (3.5-19.6%), limonene (3.4-8.4%), menthyl acetate (1.4-17.2%) and β-caryophyllene (2.4-6.3%). It is also stated that peppermint essential oil is an effective antimicrobial and pest control agent in food plants and foodstuffs (McKay and Blumberg, 2006). In addition, it has been reported that menthol is generally responsible for the antimicrobial activity of *Mentha* x *piperita* (Iscan *et al*. 2002).

The compounds known as secondary metabolites of garden sage (*S. officinalis*) are anesthetic, antihistaminic, anti-rheumatic, diuretic, expectorant, insecticide, laxative (monoterpenes); Analgesic, antiarrhythmic, antiepileptic, spasmolytic, anthelmintic, anti-inflammatory, antitumor, hypotensive and sedative properties were reported in previous studies (Abu Darwish, 2014). Some compounds of *S. officinalis* include essential oil (alpha and beta thujone, cineole, camphor, salvia tannin, etc.), flavonoids (apigenin, luteolin, genkwanin, etc.), terpenoids (ursolic acid, picrosalvin, rosmanol, saffisinolide, etc.) and phenolic acids (rosmarinic acid, chlorogenic acid, ferulic acid, caffeic acid, etc.) have been reported to be present (Ahl *et al*., 2015).

Slimani *et al*. (2022) reported that the essential oil of *L. angustifolia* contained linalool (29.95%), linalyl acetate (18.86%), *ρ*-cymene (14.68%), and α-Campholenal (10.26%). The components of lavender essential oils are grouped as terpene hydrocarbons: monoterpenes (C10), sesquiterpenes (C15), and diterpenes (C20). Antitumor activity is associated with monoterpenes. It is stated that the complex formed by linalyl acetate, alpha-terpineol, and camphor compounds found in lavender essential oil causes inhibition of the growth of human cancer cell lines. Nikšić *et al*. (2017) also reported that lavender (*L. angustifolia*) extracts, in addition to their antibacterial and antifungal effects, have potential anti-proliferative activity against many malignant cell lines (MOLT-4, MCF-7 and H460), and the greatest effect was shown on hematological malignant MOLT-4 cells. In this study, Lavandula species displayed significant antibacterial activities that might show the high potential of the compounds harbored in the lavandula species grown by Turkish farmers. These results also indicated that the extended studies should be focused on the more detailed antimicrobial, antifungal and antitumor activities of the lavandula species grown by farmers in the globally and future in Türkiye.

According to One-way ANOVA analyses, it was observed that *E. purpurea* extract caused the lowest variance among bacterial strains. In fact, this result is consistent with the fact that the effectiveness of the antimicrobial activity of *E. purpurea* observed to be quite low compared to the other plants. It is known from previous studies that extracts obtained from medicinal aromatic plants using different solvents may contain different secondary metabolites with different concentrations. However, although the chemical compositions of plants depend mainly on the plant genotype, they may differ under the influence of environmental factors such as the plant's sun exposure time, age, seedling collection method or isolation methods of extracts (Burt, 2004; Saharkhiz *et al*., 2009; Andry *et al*., 2017). It has also been reported that chemical components vary in terms of quality and yield according to plant harvest time and different parts of the plant (Hussain *et al*., 2010).

Considering the environmental conditions in which the analyzed plants were grown, it is expected that this difference will also be reflected in their cell contents. Based on this idea, whether there was a relationship between the antimicrobial activities of the secondary metabolites stored in the plants according to the geographical conditions (altitude, latitude and longitude) where they were collected was analyzed by calculating *Pearson's* correlation coefficients. The results showed that the plant extracts showed high positive correlations with altitude in application of both volumes 20 and 25 µL, and high negative correlations with latitude. Plants have high plasticity to adapt the changes in the environmental conditions. They can tolerate and adapt to eco-geographic changes in their environment by producing morphological changes up to a certain level, without changing their genetic structure (Nicotra *et al*., 2010). As the altitude increases, environmental conditions may become more challenging, such as the decrease in temperature and air humidity, increase in wind and water loss by transpiration. When environmental conditions are challenging, plants make some changes in their morphological structures and try to cope with these challenging conditions. One of the sources of the variances in the ethanol extracts of plants might be due to the differences in environmental conditions. Although the antimicrobial activities of these plant species were analyzed in this study, it can be thought that these metabolites also play an active role in the plant's defense mechanisms against the challenging environmental conditions. Additionally, different plant species may respond differently to altitude increases. Latitude is a factor related to light issue for plants. The plants are exposed to how much light during a daytime determines their photosynthesis rates. However, although there are not great differences in terms of latitude between the locations of the plants used in this study, it is estimated that they might cause significant changes in terms of plant cellular contents.

Extracting the secondary metabolites of medicinal aromatic plants with antimicrobial effects with various solvents such as ethanol, hexane, chloroform, and water is one of the popular topics recently. Some compounds found in these extracts are used in many areas such as cosmetics, medicine, paint, herbal teas, nutritional supplements, liquor, pesticides and fungicides, essential oil products, perfumes, flavoring liquids and cleaning products (Ahl *et al*. 2015). In order to benefit more efficiently in using the biologically active compounds found in natural plant extracts, the future studies should also focus on investigating the mechanisms of action and pharmacokinetics of these compounds. Even if the plants are not poisonous under normal conditions and are consumed naturally, the compounds they contain may have side effects. Therefore, it should not be forgotten that when using these plants in traditional treatments, the clinical effectiveness and safety of plant extracts and active compounds should be taken into account.

In conclusion;

- The different medicinal and aromatic plant species showed different antibacterial activities on the same bacterial strains.
- According to ANOVA and *post Hoc* tests, it was revealed that the variance in the antimicrobial activities of ethanol extracts of the different plant species among bacterial groups was significant.
- There were significant correlations between the conditions of the locations, where the plants were collected used in the study and the effectiveness of the antimicrobial activities of the plant extracts on *E. coli* and *E. faecalis* bacterial strains.
- Among the analyzed plant species, it was observed that the antimicrobial activities of *M. piperita*, *S. officinalis* and *L. angustifolia* species were significantly higher than the other species.
- The fact that *L. angustifolia*-E species gave inhibition zone diameters almost at the same rates as the positive control on *E. faecalis* bacteria showed its potential for producing natural and economical secondary metabolites with high levels of antibacterial activity that might be considered significantly for its future usage.
- As food industries tend to reduce the use of chemical preservatives in their products, essential oils of medicinal aromatic plants with potential active antimicrobial properties can be considered as a natural source to preserve or extend the shelf life of products.
- More effective secondary metabolites can be obtained from medicinal aromatic plants using different solvents and advanced extraction methods under more advanced industrial type

laboratory conditions. Since these compounds are natural, they will provide significant benefits in terms of human health and ecology.

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Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

Authorship Contribution Statement

Both authors contributed equally to the preparation of the manuscript; Investigation, Methodology, Resources, Visualization, Software, Formal Analysis, Supervision, Validation and Writing -original draft.

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