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The Relationship Between Antimicrobial Activities and Mineral Contents of Narrow Endemic Gypsophytes and Their Chemical Contents

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Abstract

Aim of study: In this study, the relationship between the antimicrobial activities and mineral contents of 7 narrow endemic gypsophyte plant species growing in extreme habitats was investigated. In addition, GC-MS analyzes of these species were also performed.

Material and methods: For this purpose, macro and micro element concentrations in soil and plant and antimicrobial activity against 13 bacteria and one yeast strain were determined by disc diffusion method in 30, 45 and 75 µL methanol and ethyl acetate extracts.

Main results: It has been presented that there is a strong antimicrobial effect potential in these gypsophyte plants. In all plant species taken, it was determined that the total Ca element among the macro elements accumulated more in the plant body than the other macro elements, and it was determined that Fe element accumulated the most among the micro elements. It was assumed that there might be a linear relationship between the strong antimicrobial activity detected in gypsophilic plant species adapted to extreme conditions and the Ca and Fe content.

Highlights: Extramophiles plants could be used in the development of antimicrobial agents in pharmaceutical industry.

Keywords: Antimicrobial Activity, Gypsopyhte, Mineral Content, Narrow Endemic

Dar Yayılışlı Endemik Jipsofitlerin Antimikrobiyal Aktiviteleri ile Mineral İçerikleri Arasındaki İlişki

Öz

Çalışmanın amacı: Bu çalışmada, ekstrem habitatlarda yetişen 7 dar yayılışlı endemik jipsofit bitki türünün antimikrobiyal aktiviteleri ile mineral içerikleri arasındaki ilişki araştırılmıştır. Ayrıca bu türlerin GC-MS analizleri de yapılmıştır.

Materyal ve yöntem: Bu amaçla, disk difüzyon yöntemiyle 30, 45 ve 75 µL'lik metanol ve etil asetat ekstraktlarında 13 bakteri ve bir maya suşuna karşı antimikrobiyal aktivite ile toprak ve bitkideki makro ve mikro element konsantrasyonları belirlendi.

Temel sonuçlar: Bu jipsofit bitkilerde güçlü bir antimikrobiyal etki potansiyeli olduğu ortaya konmuştur. Alınan tüm bitki türlerinde, makro elementlerden toplam Ca elementinin bitki gövdesinde diğer makro elementlere göre daha fazla biriktiği, ve mikro elementler arasında da en fazla Fe elementinin biriktiği belirlenmiştir. Ekstrem koşullara adapte olmuş jipsofilik bitki türlerinde tespit edilen güçlü antimikrobiyal aktivite ile Ca ve Fe içeriği arasında doğrusal bir ilişki olabileceği varsayılmıştır.

Araştırma vurguları: Ekstramofil bitkiler, ilaç endüstrisinde antimikrobiyal ajanların geliştirilmesinde kullanılabilir.

Anahtar Kelimeler: Antimikrobiyal Aktivite, Jipsofit, Mineral Konsantrasyonu, Dar Yayılışlı Endemik

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Introduction

In the treatment of diseases, antibacterial and antifungal effects of herbal preparations are very important in the development of new agents due to the increased resistance of bacteria to clinical antibiotics (Mummed et al., 2018). In some studies, there are approaches show that plants can be successful in overcoming antibiotic resistance (Celik et al., 2008; Uzel et al., 2006; Van Vuuren & Viljoen, 2011; Herken et al., 2012; Hutchings & Cock, 2018; Blonk & Cock, 2019). Therefore, the use of plant extracts as antioxidant and antimicrobial agents has been increasing in recent years (Albayrak et al., 2021).

It is known that especially endemic plant species have different antimicrobial effects (Buruk et al., 2006; Dulger, 2006; Benli et al., 2007; Türker, et al., 2009; Celik et al., 2010). Studies on antimicrobial activity are mostly concentrated on endemic plant species with a wide distribution, and studies with local endemic species are limited. The first study on the antimicrobial activities of gypsophites was presented by Ocak et al. (2021).

They have developed a set of genetic, anatomical, morphological, or metabolic mechanisms that allow plants to adapt to terrestrial environments ranging from extreme cold environments in the Arctic and Antarctic to high salinity environments, extreme temperature changes, and extreme drought conditions in desert environments in their 450 million-year evolutionary adventure (Willert et al., 1990; Alberdi et al., 2002; Amtmann et al., 2005; Celik et al., 2013; Çekiç et al., 2018; Ozdeniz, 2019).

Gypsum; it is a common soil in arid and semi-arid regions and is a physical and chemical stress factor for plant life. In gypsum soils, it prevents seedling and seed development because gypsum wraps the soil surface like a tight shell. In addition, since gypsum has low potassium (K) and magnesium (Mg) concentrations and due to the irregular uptake of nutrients from the soil by plant roots, the product yield is low and the uptake of Mg and K is inhibited when the Ca concentration is high in the relationship between macro-nutrients such as Ca, Mg, K. The Ca:Mg ratio increases in plant tissues (FAO, 1990). High calcium concentration due to the presence of gypsum may cause Ca-Mg antagonism (Özdeniz et al., 2016). Plants living in extreme habitats are collectively called extramophylls. They harbor a number of different mechanisms that enable extramophiles to withstand these extreme environments.

This study was planned by assuming that extramophiles may have developed a series of resistance mechanisms such as antimicrobial, antifungal and antiviral in the evolutionary process and that this effect may be related to the mineral content.

In this study, the relationships between the antimicrobial effects and mineral concentrations of gypsophyte species growing in gypsum soils, which are extremely arid habitats, were examined.

In this study, it was aimed to determine the antimicrobial activities of the local gypsophyte endemics *Alyssum nezaketiae* Aytaç & H. Duman., *Achillea gypsicola* Hub.-Mor., *Gypsophila germanicopolitana* Hub.-Mor., *Gypsophila simonii* Hub.-Mor., *Helianthemum germanicopolitanum* Bornm., *Onobrychis germanicopolitana* Hub.-Mor. & Simon, *Linum mucronatum* Bertol. subsp. *gypsicola* Davis extracts obtained from methanol and ethyl acetate solvent in 3 different concentrations and to reveal the relationship between the mineral content of these plants and the antimicrobial activity.

G. germanicopolitana Hub.-Mor, *G. simonii* Hub.-Mor. *A., nezaketiae* Aytaç & H. Duman., *A. gypsicola* Hub.-Mor., *H. germanicopolitanum* Bornm., *O. germanicopolitana* Hub.-Mor. & Simon ve *L. mucronatum* Bertol. subsp. *gypsicola* Davis included in the study are locally endemic species adapted only to gypsum soils.

It has been determined that the species in our study have anticancer, antibacterial, antifungal, antiviral, antioxidant and anti-inflammatory properties in various species belonging to their family. However, according to researches, it has been seen that it is used in the treatment of many diseases, including wound healing, skin disorders, digestive and respiratory tract infections, liver diseases, cardiovascular disease, cancer, diabetes, arthritis, osteoporosis, autoimmune and neurological disorders (Bouzergoune et al., 2013; Cartea et al., 2011; Erbil et al., 2015;

Grigore et al., 2020; Hussien & Aziz, 2021; Karaalp et al., 2009; Tozyılmaz et al., 2021).

Various species of the Caryophyllaceae family, especially the genus Gypsophila L., are widely used as traditional medicine by many ethnic communities around the world. Most plants of the family are used in traditional Chinese medicine (Nono et al., 2014; Mamadalieva et al., 2014; Chandra & Rawat, 2015; Sharma & Arora, 2016; Servi et al., 2019). The genus Achillea L., on the other hand, derives its name from its ancient use as a wound-healing remedy by the Trojan hero Achilles (Benedek & Kopp, 2007).

Material and Methods

Collection and Diagnosis of Plant Material

The plants constituting the study material were collected in May 2021 from the gypsum soils around Süleymanlı village, located 9-10 km south of Çankırı province. The common feature of the plants is that they are locally endemic species spreading on gypsum soils, which are extremely arid habitats for plant life (Table 1). The plant was collected as a whole and shade dried for a few weeks.

Identification of plant materials was made using Flora of Turkey and East Aegean Islands (Davis, 1965-1988). Identified specimens were controlled in the ANK herbarium, and one doublet of each plant was kept in the ANK herbarium.

Table 1. Localities of extracted plant taxa

Species	Locality/Leg.-Det./Herb.No
Achillea gypsicola Hub.-Mor.	Cankiri Ankara highway 10. km. gypsum soil, 780 m. 28.05.2021. Kurt, L., 16126
Alyssum nezaketiae Aytaç & H.Duman	Cankiri Ankara highway 9. km. gypsum soil, 695 m. 28.05.2021. Kurt, L., 16125
Gypsophila germanicopolitana Hub.-Mor.	Cankiri Ankara highway 9. km. gypsum soil, 695 m. 28.05.2021. Kurt, L., 16124
Gypsophila simonii Hub.-Mor.	Cankiri Ankara highway 9. km. gypsum soil, 695 m. 28.05.2021. Kurt, L., 16123
Helianthemum germanicopolitanum Bornm.	Cankiri Ankara highway 10. km. gypsum soil, 810 m. 28.05.2021. Kurt, L., 16121
Onobrychis germanicopolitana Hub.-Mor. & Simon	9 km south of Çankırı, above Süleymanlı village, 714 m. 28.05.2021. Kurt, L., 16122
Linum mucronatum Bertol. subsp. *gypsicola* Davis	Cankiri Ankara highway 10. km. gypsum soil, 810 m. 28.05.2021. Kurt, L., 16127

Extraction Method

The plants were washed thoroughly 2–3 times with water, and then they were air dried under shade. Afterwards, the dried plant materials were grinded in a mixer, and the powder was kept in the brown glass bottle with paper labeling. Between the range of 10–30 g, grinded materials were extracted with 250 mL of methanol and ethyl acetate (chosen as solvents of different polarity) in a soxhlet apparatus by continuous heat extraction for 24 h. All extract solutions were filtered through Whatman No. 1 paper. Then, filtrates were evaporated with rotary evaporator. The filtrates were concentrated to a small volume under reduced pressure and evaporated to dryness. The extracts were stored in refrigerator at about 4 °C after sealed with paraffin in order to use in further studies.

Microorganism Strains

The thirteen bacteria strains *Enterobacter aerogenes* ATCC 13048, *Klebsiella pneumoniae*, *Pseudomonas fluorescens*, *Salmonella kentucky*, *Enterococcus faecalis* ATCC 29212, *Listeria innocua*, *Salmonella typhimurium* SL1344, *Enterococcus faecium*, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* DSMZ 20044, *Bacillus subtilis* DSMZ 1971, *Escherichia coli* ATCC 25922, *Serratia marrescens* and one yeast strains *Candida albicans* ATCC 10231 were used to the antimicrobial activity test.

Disk Diffusion Test

A disk diffusion method described in the literature (Andrews, 2005) was used to

determine the antimicrobial potentials of our samples. 90 mm diameter Petri dishes containing 20 mL of Mueller-Hinton agar were used to standardize the study as described in several previous studies (Özkan et al., 2018). The plant extracts were dissolved in methanol to reach a concentration of 10 mg/mL. After loading 30, 45, and 75 µL of each extract on sterile blank discs, a total of 0.3 mg, 0.45 mg, and 0.75 mg plant extracts were applied to sterilized 6 mm diameter paper disks. The disks were allowed to dry overnight at 25°C under sterile conditions to evaporate the residual solvent.

To prepare microorganism suspensions with a bacterial concentration in 0.9% sterile saline solution, they were adjusted to 0.5 McFarland (1-2 x 10^8 CFU/mL) turbidity standards with a densitometer (DEN-1B, Biosan, Riga, Latvia). These bacterial suspensions are spread on the surfaces of Mueller-Hinton agar plates. Then, they were kept in aseptic conditions for 5 minutes. After the negative control and sample disks were placed on the surface of the same inoculated Petri dish (Mueller-Hinton Agar), the plates were incubated at 37°C for 24 hours. Finally, after one day, the inhibition zone diameters including the disc were measured in mm and this process was repeated three times.

Mineral Element Concentrations of Plant Samples and Soil Samples

Plant samples, which were collected from Çankırı district, were washed with deionized water then were dried in a thermos-ventilated oven at 65°C for three days. For elemental analysis all plant samples were grinded and digested with concentrated nitric acid (65% Merck) using a microwave digestion system (Berghof-MWS-2, 24 units). Total K, Ca, Mg, P, Fe, Zn, Cu and Mn concentrations were determined by ICP-OES (Perkin Elmer Optima 2100 DV, Waltham, MA, USA). The peach leaves received from National Institue of Standart and Technolgy were used as the standart reference material with the code NIST-1547.

Soil samples were taken simultaneously from the place where the plant samples were collected. Exchangeable K, Ca, Mg (Pratt, 1965) extractable Fe, Zn, Cu and Mn (Lindsay & Norwell, 1978) concentrations were determined by ICP-OES (Perkin Elmer Optima 2100 DV, Waltham, MA, USA).

GC-MS Analysis

The plant extracts were diluted with the same solvent used in the extraction process for volatile component analysis. The methyl esterification method was used to determine the fatty acid profiles of the extracts. For this purpose, 2 N methanolic KOH was added to the samples treated with n-hexane. Both analyzes were performed using Gas Chromatography- Mass Spectrometry (Shimadzu GC-MS QP 2010 ULTRA). The analyzes was carried out using a capillary column (RTX 5MS; 30 m; 0.25 mm; 0.25 µm) with helium as the carrier gas. The interface, ion source, and injection temperatures were adjusted at 250°C, 200°C, and 250°C, respectively. The column temperatures were 40°C and 90°C for volatile compound and FAME analyzes, respectively. The injection volume was 1 µL, and the injection was performed using the 1:5 split ratio. During the volatile compound analysis, a furnace cycle of 3 minutes at 40°C followed by a 4°C/min rise from 40°C to 240°C and furnace cycle of 5 minutes at 90°C followed by a 4°C/min rise from 90°C to 250°C, 5 min at 250°C for FAME analyses were used. The peaks in the chromatograms were compared with the W9N11 library for the identification of all components.

Results and Discussion

GC-MS Results

As a result of the volatile component analysis of 7 narrow endemic gypsophyte species extracted with methanol and ethyl acetate, different main components were obtained depending on the solvents.

Mome inositol was determined as the main component of methanolic extracts of plants at a rate of 35.52%, 34.04%, 49.07% and 16.89% for *H. germanicopolitana*, *G. germanicopolitana*, *G. simonii* and *O. germanicopolitana*, respectively.

42.58% grossmysine was found as the most abundant compound in the 6 methanolic extracts. 1-Nonadecene was highly detected in ethyl acetate extractions of all plants.

High levels of alpha tocopherol (11.93%) were determined in the ethyl acetate

extraction of narrow endemic 7 gypsophyte plants. The main components of the plant extracts are given in Table 2. Heneicosanoicacid 20-methyl; 9,12,15-Octadecatrienoic acid (Z, Z, Z). The most abundant fatty acids in methanol and ethyl acetate extracts were found to be 9,12-Octadecadienoic acid (Z, Z) and Hexadecanoic acid.

Unlike other plants, 12-hydroxy-9-cis-octadecenoic acid (Ricinoleic acid) detected in both solvent extracts of *L. mucronatum* subsp. *gypsicola*. The fatty acid profile of the plant extracts is given in Table 2.

Some non-fatty components were also found in the fatty acid analysis of plant extracts. Hexatriakontan, ethyl acetate extract of *A. nezaketiae* (37.22%), ethyl acetate extract of *G. simoni* (16.32%), and methanolic extract (17.94%). *L. mucronatum subsp. gypsicola* 's ethyl acetate extract (30.04%) and methanolic extract (40.85%) were detected at high rates. Tetradecanal, Pentatriacontane, Octatriacontyl pentafluoropropionate and Heptadecanal were also found in *H. germanicopolitana*, *G. germanicopolitana*, *G. simonii*, *A. nezaketiae* and *A. gypsicola*, respectively.

Table 2. The fatty acid profile of plant extracts

Plant Taxa	Macrocomponents						
H. germanicopolitana	%35,52 Mome inositol	% 15,11 Phytol	% 8,55 Neophytadiene	% 3,47 Hexahydro Farnesyl Acetone			
G. germanicopolitana	% 34,04 Mome inositol	% 4,58 DL-β Phenyllactic acid	% 4,03 (-)-Loliolide	% 3,48 Iso-Amyl Phenyl Acetate			
G. simonii	% 49,07 Mome inositol	% 5,65 2-(Benzyloxy)-5-(2-Nitrovinyl) Anisole	% 4,30 L-Chlorononane	% 3,81 Blumenol B	% 3,01 Coumaran	% 2,64 6-Ethoxy-6-Methyl-2-Cyclohenanone	
O. germanicopolitana	% 27,73 Neophytadiene	% 6,89 Mome inositol 1	% 11,14 DL-β Phenyllactic acid	% 3,1 Iso-Amyl Acetate			
A. nezaketiae	% 12,27 Roughanic acid	% 4,05 Octacosyl acetate	% 3,35 N-Formyl-DL-Valine	% 2,57 Lauric acid			
A.gypsicola	% 42,58 Grossmisine	% 2,33 Scoparone					
L. mucronatum subsp. *gypsicola*	% 7,92 1,6 Anhydro Beta-D-Glucopyranose,	% 7,90 3-Deoxy-D-Mannoic Lactone	% 5,25 Iso-Amyl Acetate	% 4,79 Guaiacol	% 4,68 Guanosine	% 3,64 Allo inositols	% 3,23 Phytol

Antimicrobial Activity Results

Inhibition zone diameter data from the disk diffusion test are shown in Tables 3 and 4. Negative controls show no activity according to the results. In addition, statistical analysis showed that the differences between the results of three replicates of each extract volume were not statistically significant ($p>0.05$).

Table 3 clearly shows that 30 µL methanol extract of plants were presented antimicrobial activity against bacteria except for *Listeria innocua* and *Candida albicans* with inhibition zones between 7 and 14 mm. 45 µL methanol extract of plants offered antimicrobial activity against all microorganisms observed in 30 µL, with zones of inhibition ranging from 7 to 20 mm. 75 µL methanol extract of plants offered antimicrobial activity against all microorganisms observed in 30 µL, with zones of inhibition ranging from 8 to 24 mm. However, methanol extracts of plants showed weak antimicrobial activity against *Enterococcus faecalis*, *Listeria innocua* and

Candida albicans with 7 and 15 mm zones of inhibition.

Table 4 shows that 30 µL ethyl acetate extract of the plants confers weak activity against bacteria except for *Staphylococus aureus* with inhibition zones between 7 and 15 mm. 45 µL ethyl acetate extract of plants were presented antimicrobial activity against some microorganisms observed in 30 µL with inhibition zones ranging between 7 and 16 mm. 75 µL of methanol extract of plants offered antimicrobial activity against some microorganisms observed in 30 µL, with zones of inhibition ranging from 8 to 24 mm.

Methanol extracts of plants showed weak antimicrobial activity against *Enterococcus faecalis*, *Listeria innocua* and *Candida albicans* with zones of inhibition from 8 and 17 mm. However, all methanol extract of plants showed strong antimicrobial activity against *Staphylococcus aureus* and *Serratia marrescens* with zones of inhibition of 7 and 20 mm.

Table 3. Antimicrobial activity results for methanol etracts (mm)

Plant extracts	µL	Microorganisms													
		Enterobacter aerogenes	*Klebsiella pneumoniae*	*Pseudomonas fluorescens*	*Salmonella kentucky*	*Enterococcus faecalis*	*Listeria innocua*	*Salmonella typhimurium*	*Candida albicans*	*Enterococcus faecium*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Escherichia coli*	*Serratia marrescens*
Helianthemum germanicopolitana	30	10	10,5	7	9,5	7	-*	10	-	10,5	14	12	7	10,5	10
	45	12,5	13,5	7,5	11,5	8	8	13	7,5	12,5	18,5	14	9	12,5	11
	75	15,5	15	9	14	9	9	15	9	15	23	16,5	11	15	14
Gypsophila germanicopolitana	30	8,5	9,5	7	9	-	-	9	-	8	13	10	7	11	7
	45	9,5	11	8	11	7	7	11	7	11	19	12	8	13	9
	75	11,5	12,5	10	12,5	10	9	13	9	13	21	14	10	15	13
Gypsophila simonii	30	10	9,5	7	7	-	-	8	-	7	7	7	7	7	7
	45	13	12	8	10	-	10	10	7	10	10	9	7	11	9
	75	14	13	9	12	7	11	13	8	11	13	11	8	12	12
Onobrychis germanicopolitana	30	8	8	8	9	-	7	10	7	9	12	9	8	11	10
	45	10	10	10	11	-	8	12	8	11	14	10	10	12	13
	75	13	13	12	13	10	11	14	9	13	16	13	14	15	15
Alyssum nezaketiae	30	10	8	8	9	7	7	9	-	8	14	7	7	8	7
	45	11	10	9	11	8	8	11	7	10	15	8	8	10	9
	75	13	13	11	12	12	10	14	8	12	19	11	10	12	11
Achillea gypsicola	30	9	11	10	7	-	-	9	10	8	9	10	7	9	10
	45	11	12	13	11	-	-	10	13	10	10	11	8	10	11
	75	14	15	15	12	9	9	12	15	12	12	13	13	12	14
Linum mucronatum subsp. *gypsicola*	30	10	10	8	10	7	7	11	7	11	19	10	7	10	11
	45	11	11	10	12	8	8	13	9	13	20	12	9	12	13
	75	13	13	11	15	11	13	16	11	15	24	15	12	13	14
Ciprofloxacin***		30	30	19	34	19	18	35	-	29	22	34	36	-	nt**

(*: No inhibition, **: not tested, ***: Standard antibiotic(5**µg**)

Table 4. Antimicrobial activity results for ethyl acetate extracts (mm)

Plant extracts	µL	Microorganisms													
		Enterobacter aerogenes	*Klebsiella pneumoniae*	*Pseudomonas fluorescens*	*Salmonella kentucky*	*Enterococcus faecalis*	*Listeria innocua*	*Salmonella typhimurium*	*Candida albicans*	*Enterococcus faecium*	*Staphylococcus aureus*	*Staphylococcus epidermidis*	*Bacillus subtilis*	*Escherichia coli*	*Serratia marrescens*
Helianthemum germanicopolitana	30	10	11	7	10	7	7	13	-*	9	15	11	7	12	15
	45	12	13	8	12	9	8	15	7	10	20	12	9	14	18
	75	13,5	15	10	15	12	10	19	9	12	25	14	11	15	20
Gypsophila germanicopolitana	30	10	11	7	-	7	-	9	-	9	13	9	7	12	15
	45	11	13	8	9	9	-	10	7	10	16	10	9	14	16
	75	13	15	10	11	11	9	13	8	13	20	13	12	15	17
Gypsophila simonii	30	-	8	7	7	-	-	10	10	8	9	15	-	9	15
	45	9	10	8	8	-	7	11	12	10	11	16	7	11	20
	75	10	13	9	10	8	9	13	15	13	13	17	8	13	24
Onobrychis germanicopolitana	30	9	9	-	7	-	-	10	7	7	10	7	9	9	13
	45	11	11	7	9	7	-	12	9	9	11	9	10	10	14
	75	12	12	9	13	8	7	14	11	10	15	13	11	11	15
Alyssum nezaketiae	30	9	8	10	7	-	7	-	-	7	13	9	7	9	13
	45	11	11	11	11	7	8	9	7	9	20	11	8	11	15
	75	13	13	13	14	9	9	11	9	12	22	14	11	13	18
Achillea gypsicola	30	-	-	-	-	-	-	-	-	-	7	-	-	-	-
	45	-	-	-	-	-	-	-	-	-	9	-	-	-	-
	75	7	9	-	-	-	8	9	-	8	10	-	8	7	11
Linum mucronatum subsp. *gypsicola*	30	9	9	7	9	-	7	9	-	11	15	10	8	10	10
	45	10	11	8	10	-	8	11	7	12	17	11	11	11	11
	75	11	13	9	12	8	9	12	8	13	20	12	12	12	12
Ciprofloxacin***		30	30	19	34	19	18	35	-	29	22	34	36	-	nt**

(*: No inhibition, **: not tested, ***: Standard antibiotic (5**µg**))

Mineral Element Concentration Results

Macro and micro element concentrations of soil sample

In the soil sample taken to represent the area, it was detected that the exchangeable K and Mg concentration is sufficient, the Ca concentration is high, extractable Fe concentration is high, the Zn and Mn concentrations are very low, and the Cu concentration is sufficient (FAO, 1990) (Table 5).

Table 5. Concentrations of plant available macro and micro elements in soil

	K	Ca	Mg	Fe	Zn	Cu	Mn
Soil	mg kg^{-1}						
	235	30568	248	6.84	0,11	1.33	3.03

Total macro and micro element concentrations of plant species

It was seen that the total K concentrations of the plant species varied between 3.62-36.9 g kg^{-1}, and the lowest K concentration was in *A. gypsicola*, and the highest total K concentration was in *G. simonii*. Ca concentrations are in the range of 12.7-75.8 g kg^{-1} and the lowest total Ca concentration is in *L. micronatum ssp*. *gypsicola* species, the highest in *A. gypsicola* species. The Mg concentrations are in the range of 2.03-31.6 g kg^{-1} and the lowest total Mg concentration is in *L. micronatum ssp. gypsicola* species, the highest in *G. simonii* species. The P concentrations of the plant species were in the range of 0.41-2.24 g kg^{-1}, and the lowest total P concentration was found in *A. gypsicola* and the highest in *A. nezaketiae* (Figure 1). It was concluded that in all plant species, the total Ca element accumulates more in the plant tissue than the other macro elements (Figure 1).

It was concluded that the total Fe concentrations of the plant species varied between 98.0—3003 mg kg^{-1}, and the lowest Fe concentration was in *G. germanicopolitana*, while the highest total Fe concentration was in *A. gypsicola*. The Zn concentrations were in the range of 3.83-36.2 mg kg^{-1}, the lowest Zn concentration was in *O. germanicopolitana* species, the highest was *L. micronatum* ssp. *gypsicola* species, the Cu concentrations were in the range of 0.56—9.27 mg kg^{-1}, the lowest Cu concentration was in *G. simonii* species, and the highest in *H. germanicopolitana* species.

The total Mn concentrations of the plant species were in the range of 14.8-55.2 g kg^{-1} and the lowest Mn concentration was in *G. germanicopolitana* and the highest in *A. gypsicola* (Figure 2).

These results showed that most Fe element among the microelements was accumulated in plant species. (Figure 2).

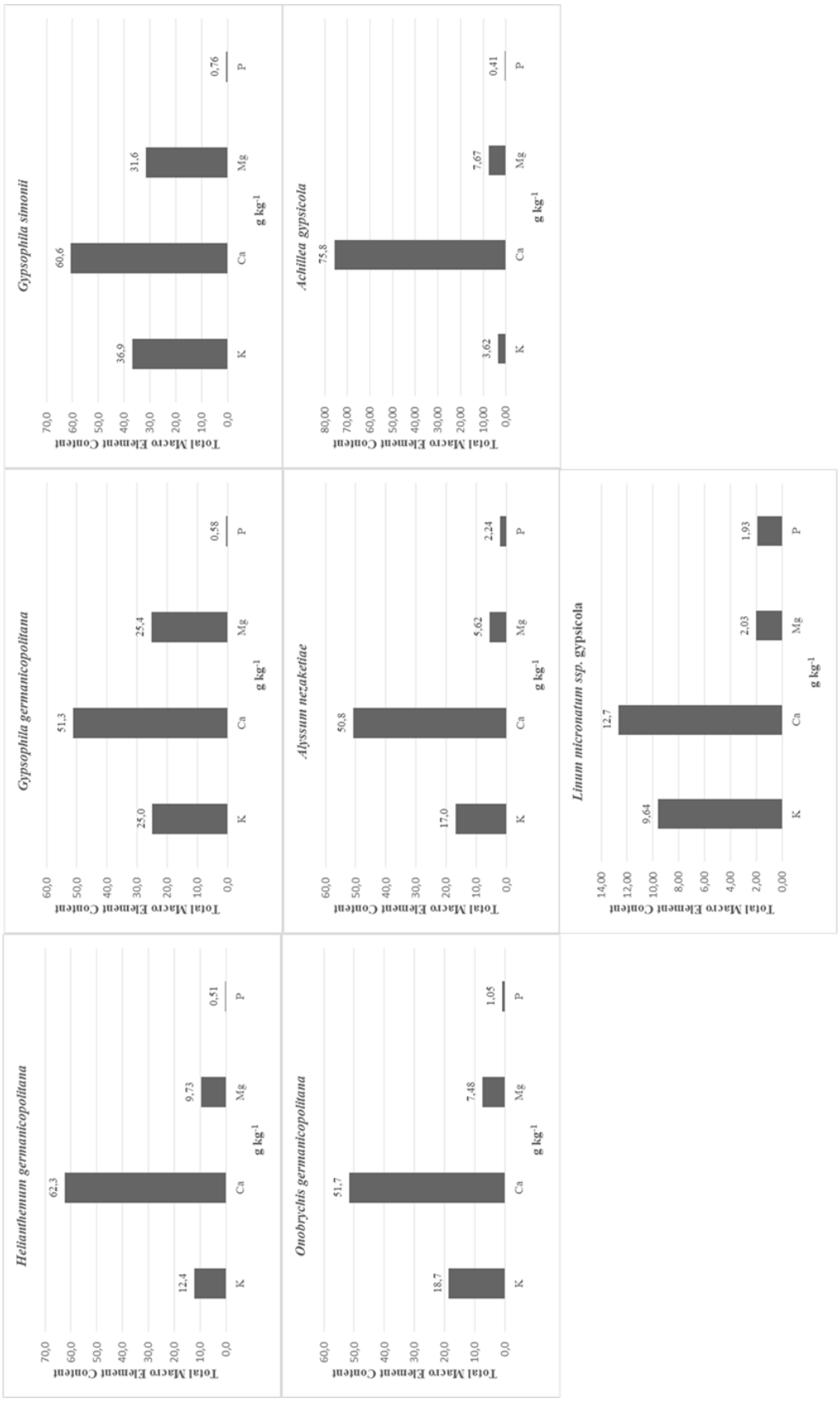

Figure 1. Macro element concentrations of plant samples

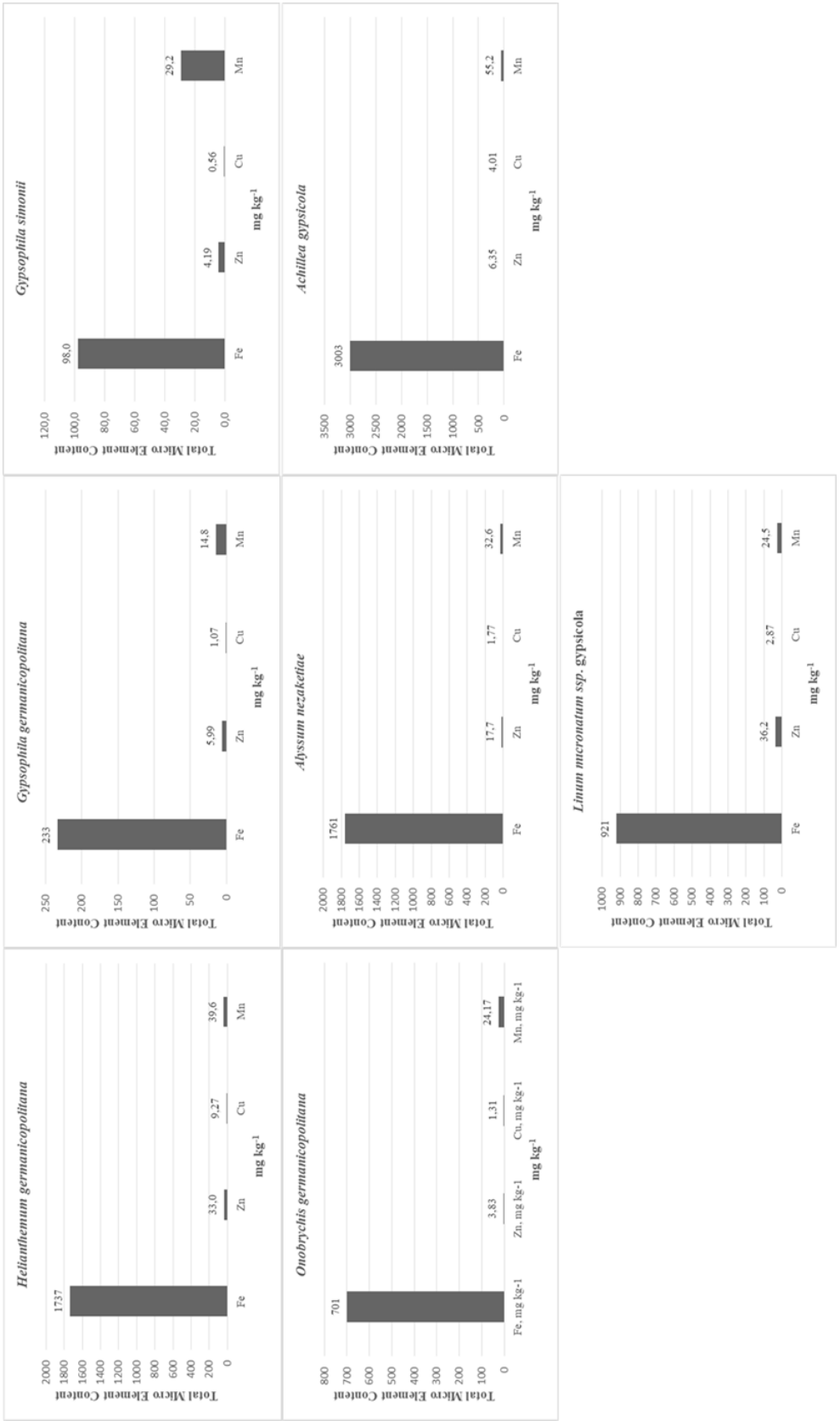

Figure 2. Total micro element concentrations of plant species

Conclusion

In this study, it was aimed to investigate the antimicrobial activities of the extracts of 7 locally gypsophyte endemic plant species, called extramophytes, in 3 different concentrations obtained from methanol and ethyl acetate solvent on 13 bacteria and a yeast strain, and to reveal the relationship between the mineral content of these plants and antimicrobial activity. For this purpose, macro and micro element concentrations in and plant and antimicrobial activity in 13 bacteria and one yeast strain were determined by disc diffusion method in 30, 45 and 75 µL methanol and ethyl acetate extracts.

Inhibition zone diameters obtained from the disk diffusion test were 7 to 14 mm in 30 µL of methanol extract, 45 µL of plant methanol extract with inhibition zones between 7 and 20 mm and 75 µL of methanol extract, inhibition zones between 8 and 24 mm showed microorganisms exhibited potent antimicrobial activity against. 30 µL ethyl acetate extract of plants between 7 and 15 mm, 45 µL of plants between 7 and 16 mm, and 75 µL of methanol extract between 8 and 24 mm presented strong antimicrobial activity.

When the activity values obtained from this study are compared with the values available in the published literature for similar plants, it is seen that the antimicrobial properties of gypsophilic plants are more effective (Buruk et al., 2006; Servi et al., 2019; Ocak et al., 2021). In all plant species, the Ca element among the macro elements accumulated more in the plant tissue, and it was also determined that the Fe element accumulated the most among the microelements.

When compared with some studies on mineral content and antimicrobial activity (Tabanca et al., 2006; Imelouane et al., 2011; Salleh et al., 2011; Erden et al., 2013), similar results are seen. This case might be stemming from a linear relationship between the strong antimicrobial activity detected in gypsophilic plant species adapted to extreme conditions and the Ca and Fe concentration.

The most important result reached in this study is that there is a linear relationship between antimicrobial activity and mineral content in 7 local gypsophyte endemic plant species, as well as the potential to be used by the pharmaceutical industry as the antimicrobial activities of its extracts are very high on 13 bacteria and one yeast strain.

Ethics Committee Approval

N/A

Peer-review

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Author Contributions

Conceptualization: E.Ö., L.K.; Investigation: E.Ö., L.K.; Material and Methodology: S.T., H.A., K.G., M.G., O.E.Ö., F.K.; Supervision: E.Ö., L.K., M.G., S.T.; Visualization: H.A., O.E.Ö., F.K.; Writing-Original Draft: E.Ö., L.K.; Writing-review & Editing: E.Ö., H.A., K.G., M.G., L.K.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The authors have no conflicts of interest to declare.

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