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RESEARCH ARTICLE



Antioxidant and antimicrobial activities of plant flowers growing in distinct geological habitats (serpentine, gypsum, and limestone)

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ABSTRACT

In this study, the flowers of 20 plant species [*Gypsum* (*Linum flavum*, *Paracaryum stenolophum*, *Onobrychis nitida*, *Gypsophila lepidioides*, *Psephellus recepii*, *Tanacetum heterotomum*, *Verbascum alyssifolium*, limestone (*Teucrium leucophyllum*, *Stachys sparsipilosa*, *Phlomis oppositiflora*, *Pelargonium endlicherianum*, *Chrysophthalmum montanum*, serpentine (*Convolvulus pseudoscammania*, *Fumana aciphylla*, *Gladiolus halophilus*, *Hypericum thymbrifolium*, *Salvia indica*, *Artemisia squamata*, *Erysimum pulchellum*, *Consolida olopetala*], including 11 endemic species, growing in various habitats (gypsum, limestone, and serpentine) in Erzincan province were examined. By focusing on the antioxidant capacities and antibacterial activities of these species, the study aims to elucidate the effects of these ecosystems on the biosynthesis of plant secondary metabolites, primarily phenolic compounds and flavonoids, and their adaptive biochemical responses to unique environmental conditions. Antioxidant properties were assessed using DPPH, FRAP, TPC, and TFC assays. Results showed significant differences in antioxidant compound concentrations among plants from different habitats, with the highest levels in serpentine and gypsum regions. *Artemisia squamata* L. from serpentine had the strongest DPPH activity (24.83 ± 1.25 µg/ml), *Erysimum pulchellum* had the highest TPC (89.44 ± 0.76 mg GAE/g), and *Tanacetum heterotomum* had the highest TFC (43.48 ± 2.08 mg QE/g) in gypsum. FRAP activity was highest in serpentine species, particularly *Consolida olopetala* (32.14 ± 1.84 mg TE/g). Furthermore, Antibacterial assays against eight ATCC strains revealed significant inhibitory effects by *Stachys sparsipilosa*, *Tanacetum heterotomum*, *Teucrium leucophyllum*, and *Verbascum alyssifolium* against *Enterococcus faecium* and *Salmonella enteritidis* with *Teucrium leucophyllum* showing the highest activity (MIC of 31.25 µg/ml against *Salmonella enteritidis*). The study highlights

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the influence of habitat on the biosynthesis of bioactive compounds, suggesting that plants from serpentine and gypsum soils have enhanced antioxidant and antimicrobial properties, making them potential candidates for natural therapeutic agents.

Abbreviations: TPC: total phenolic contents; TFC: Total flavonoid contents; DPPH: 2,2-diphenyl-1-picrylhydrazyl; FRAP: Ferric reducing antioxidant power; MIC: Minimum inhibitory concentration

Introduction

Throughout history, plants have been crucial in nutrition and medicine, aiding in disease prevention and treatment. Initially, the utilisation of plants was driven by basic survival instincts. Over time, however, humans began to harness both biotic and abiotic components from their surroundings, integrating them into increasingly sophisticated medical practices. Given that plants harbour a multitude of bioactive compounds with significant health benefits, contemporary applications of plants are increasingly informed by empirical knowledge and scientific research. This evolution marks a transition from rudimentary survival strategies to a sophisticated comprehension of their therapeutic properties (Yücel and Tülükoğlu 2000; Tulukcu and Sağdıç 2011; Adem et al. 2019).

Plants synthesise a remarkable array of bioactive compounds essential for their adaptation and survival in various environmental contexts. Among these, secondary metabolites such as polyphenols, flavonoids, and antioxidants play pivotal roles in plant defense mechanisms, food, medicine, and industry and confer significant health benefits to humans (Rao and Ravishankar 2002; Demirtas et al. 2017; Köksal et al. 2021; Bilginer et al. 2022). The biochemical profiles of plants are influenced by a combination of genetic and environmental factors, including climate, soil composition, and various stressors (Lin and Lai 2006). Specialised soil habitats, such as serpentine, gypsum, and limestone, create unique ecological conditions that significantly affect the chemical composition of plants, particularly in terms of secondary metabolite production (Rajakaruna and Boyd 2014). These distinctive habitats exert substantial selective pressures, driving the evolution of diverse biochemical adaptations that reflect a sophisticated interplay between soil chemistry and plant metabolism. Studying the phytochemical characteristics of plant species from these regions is essential to understanding how environmental factors affect plant chemistry.

Serpentine soils are characterised by high concentrations of heavy metals such as nickel, chromium, and cobalt (Ni, Cr, Co), and low levels of essential nutrients like calcium, nitrogen, phosphorus, and potassium (Ca, P, N, K), present extreme challenges for plant growth (Michalak 2006; Gall et al. 2015; Zlatić et al. 2017). Plants inhabiting these soils have evolved unique adaptive mechanisms, including the production of elevated levels of antioxidant compounds to counter oxidative stress resulting from heavy metal toxicity (Proctor 1971; Cekic et al. 2021). These antioxidants, by neutralising reactive oxygen species, are essential for maintaining cellular integrity under harsh conditions, underscoring the importance of biochemical resilience for plant survival (Shao et al. 2008; Abdulfatah 2022). Research indicates that the phytochemical profiles of serpentine flora are significantly impacted by the soil's unique chemical properties (Cekic

et al. 2021). Elevated heavy metal levels in these soils promote the synthesis of specialised secondary metabolites that play roles not only in detoxification but also in growth regulation and pathogen resistance, showcasing a dynamic relationship between environmental chemistry and plant biochemistry.

Similarly, gypsum soils present distinct challenges due to their high calcium sulphate content. This specific soil composition not only influences plant physiology and biochemistry but also enhances the activity of antioxidant enzymes and the synthesis of secondary metabolites, contributing to improved resilience against environmental stressors such as drought (Shafiq et al. 2021). Calcium, the dominant element in gypsum soils, stabilises plant cell membranes, protecting them against oxidative stress, which is critical for maintaining cellular integrity under adverse conditions (Luo et al. 2011). The elevated levels of polyphenols and flavonoids observed in plants from gypsum habitats reflect an adaptive response to environmental stressors, where increased secondary metabolite synthesis bolsters plant survival and fitness (Jimoh et al. 2019). The specific chemical environment of gypsum soils stimulates certain metabolic pathways, leading to an accumulation of bioactive compounds essential for plant defense. These compounds not only contribute to stress adaptation but also play a significant role in ecological interactions, enhancing plants' resilience against herbivores, pathogens, and other environmental pressures.

In contrast, limestone habitats, characterised by their high calcium carbonate content and alkaline pH levels, create conditions that limit the availability of essential nutrients such as iron, magnesium, and phosphorus, necessitating specialised enzymatic adaptations in plants (Hussein et al. 2023). The alkaline environment and nutrient limitations in limestone soils prompt plants to modify root exudation and activate specific ion transporters, facilitating nutrient acquisition and promoting biochemical adjustments that shape their phytochemical profiles. Unlike serpentine and gypsum soils, which are distinguished by high levels of heavy metals and sulphate content, respectively, limestone soils typically produce plants with lower concentrations of certain secondary metabolites such as alkaloids, flavonoids, and phenolics (Varol et al. 2023). This variation in secondary metabolite levels underscores the complexity of habitat-specific biochemical adaptations, illustrating the unique ways in which soil types can influence plant chemistry.

The study of biological activities in plants adapted to serpentine, gypsum, and limestone soils holds significant ecological, medicinal, and biotechnological importance. These unique soil types impose distinct chemical and physical constraints that shape the secondary metabolite profiles of plants, often leading to the development of specialised biochemical adaptations, such as elevated antioxidant and antimicrobial compounds, to counteract environmental stressors. This adaptation process provides insights into plant defense mechanisms and has the potential to unveil novel bioactive compounds with therapeutic applications. Within this context, investigating the antioxidant and antimicrobial properties of floral parts is particularly significant due to their distinct biochemical makeup and ecological functions. Flowers are rich in diverse secondary metabolites, such as flavonoids, phenolic acids, and essential oils, which protect reproductive structures from microbial invasion and oxidative damage, aid in attracting pollinators, and defend against herbivores (Lucas-Barbosa et al. 2016; Hyeon et al. 2021; Pei et al. 2023; Zhang et al. 2023). These bioactive compounds are often more concentrated in flowers than in other plant tissues, positioning floral extracts as a promising source of natural antioxidants and antimicrobials with applications in

pharmaceuticals, food preservation, and cosmetics (de Menezes Filho et al. 2022; Tungmunnithum et al. 2022). Thus, the exploration of flowers not only enhances our ecological understanding but also provides valuable bioactive agents potentially less prevalent in other plant parts.

The objective of this study is to investigate the influence of environmental factors specifically serpentine, gypsum, and limestone ecosystems on the biosynthesis of antioxidant and antimicrobial compounds in the flowers of 20 plant species, 11 of which are endemic. This research aims to elucidate the defense mechanisms developed by these species in response to environmental stress, with a particular focus on the floral parts, which have been largely overlooked in previous studies. Given the promising bioactive potential of these plants, there exists a significant gap in the literature regarding both their bioactive properties and the impact of varying environmental conditions on these properties. By focusing specifically on the flowers, this study seeks to address this gap, providing valuable insights into novel antioxidant and antimicrobial compounds and their ecological relevance. This study constitutes the first comprehensive examination of the antioxidant and antibacterial properties inherent in the floral components of these plants. When examining these plants as a whole, according to the literature, there are no studies on both the antioxidant and antimicrobial properties of *Linum flavum*, *Paracaryum stenolophum*, and *Onobrychis nitida*. In contrast, *Gypsophila lepidioides*, *Psephellus recepii*, *Tanacetum heterotomum*, *Verbascum alyssifolium*, *Teucrium leucophyllum*, *Phlomis oppositiflora*, *Chrysophthalmum montanum*, *Convolvulus pseudoscammania*, *Fumana aciphylla*, *Gladiolus halophilus*, and *Salvia indica* exhibit antioxidant potential without corresponding antimicrobial studies (Varol et al. 2023). Meanwhile, *Pelargonium endlicherianum* and *Hypericum thymbrifolium* demonstrate both antioxidant and antimicrobial properties (Ozbilge et al. 2010; Şeker Karatoprak et al. 2017). Additionally, *Stachys sparsipilosa*, *Artedia squamata*, *Erysimum pulchellum*, and *Consolida olopetala* have not been sufficiently investigated in these aspects. These findings have broad implications, including the potential development of plant-derived agents for agriculture and healthcare, the promotion of sustainable farming practices, and the discovery of new therapeutic applications. Additionally, focusing on plants adapted to local soil environments contributes to biodiversity conservation by underscoring the ecological significance of flora in extreme habitats. Thus, the investigation of antioxidant and antimicrobial properties in plants from diverse soil types not only enriches the understanding of plant ecology and evolutionary biology but also opens avenues for applications in medicine, agriculture, and biotechnology.

Material and methods

Plant material

The completely white and crystallised areas located at the 3rd km from the end of Kemah-İliç highway towards Yahşiler Village, the limestone rocks of Kemaliye Ocak Village and Eriç Village, and the serpentine areas around Kemah Yücebelen Village were selected as the study area. For this study, 20 plant species (11 of which are endemic) growing in serpentine, gypsum, and limestone regions were selected. [Gypsum (*Linum flavum* L. (Herbarium number: EBYU-0000010), *Paracaryum stenolophum* Boiss. (Herbarium number: EBYU-0000011), *Onobrychis nitida* Boiss. (Herbarium number: EBYU-

0000012, endemic), *Gypsophila lepidioides* Boiss. (Herbarium number: EBYU-0000013, endemic), *Psephellus recepii* Wagenitz & Kandemir (Herbarium number: EBYU-0000014, endemic), *Tanacetum heterotomum* (Bornm.) Grierson (Herbarium number: EBYU-0000015, endemic), *Verbascum alyssifolium* Boiss (Herbarium number: EBYU-0000016, endemic), limestone (*Teucrium leucophyllum* Montbret & Aucher ex Benth (Herbarium number: EBYU-0000017, endemic), *Stachys sparsipilosa* R.Bhattacharjee & Hub-Mor. (Herbarium number: EBYU-0000018, endemic), *Phlomis oppositiflora* (Boiss. & Hausskn) (Herbarium number: EBYU-0000019, endemic), *Pelargonium endlicherianum* Fenzl. (Herbarium number: EBYU-0000020), *Chrysophthalmum montanum* (DC.) Boiss. (Herbarium number: EBYU-0000021), serpentine (*Convolvulus pseudoscammania* C. Koch. (Herbarium number: EBYU-0000022, endemic), *Fumana aciphylla* Boiss. (Herbarium number: EBYU-0000023), *Gladiolus halophilus* Boiss. & Heldr. (Herbarium number: EBYU-0000024, endemic), *Hypericum thymbrifolium* Boiss & Noé (Herbarium number: EBYU-0000025, endemic), *Salvia indica* L. (Herbarium number: EBYU-0000026), *Artemisia squamata* L. (Herbarium number: EBYU-0000027), *Erysimum pulchellum* Willd (Herbarium number: EBYU-0000028), *Consolida olopetala* (Boiss) Hayek (Herbarium number: EBYU-0000029)]. The plant samples were authenticated by Prof. Dr. Ali KANDEMİR (Erzincan Binali Yıldırım University, Faculty of Art and Sciences, Department of Biology). All plant materials examined in the study were collected from existing natural populations in the field and have been preserved in the Herbarium of Erzincan Binali Yıldırım University (EBYU). No artificial propagation methods, such as clonal or seed-based breeding, were employed for any of the samples. Each plant species was represented by mature individuals prevalent in the region. These samples, although varying in maturity levels depending on the species' natural habitats, were carefully selected to fully exhibit the typical morphological characteristics of each species.

Extraction procedure

The floral parts of the plants were initially air-dried at ambient temperature to ensure the preservation of their bioactive compounds. Subsequently, the dried materials were finely ground into a powder. A 10 g sample of this powdered material was subjected to maceration overnight in a 100 ml mixture of ethanol–water (7:3, v/v). The ethanol–water mixture was selected for its efficacy in extracting both polar and semi-polar components. Furthermore, the use of ethanol–water mixtures is well-documented in traditional medicine, where such solvents are frequently employed for the extraction of medicinal plants (Cerulli et al. 2021; Gözcü et al. 2024b). This extraction process was performed in triplicate to ensure reproducibility and accuracy of the results. Following the extraction, the plant residues were carefully removed, and the combined solvents were subjected to evaporation to eliminate the ethanol. The remaining aqueous phase was then lyophilised, resulting in the formation of an amorphous solid. The obtained extracts were stored in dark flasks at a temperature of +4°C to maintain their stability and prevent degradation until further use.

DPPH free radical scavenging activity

The free radical scavenging activities of the extracts were assessed using a modified method by (Gözcü et al. 2024a). Initially, a 0.26 mM DPPH solution and extract stock

solutions (1 mg/ml) were prepared and diluted to various concentrations (20–1000 µg/ml) in ethanol. Each diluted extract was mixed with 1 ml of the DPPH solution, vortexed, and incubated in the dark for 30 min at room temperature. Absorbance was measured at 517 nm, and percentage scavenging activity was calculated. Finally, IC₅₀ values were determined and compared to Trolox, Ascorbic acid, BHA, and BHT to evaluate antioxidant efficacy.

Ferric-reducing power activity (FRAP)

The reducing power activity assay of the plant materials was conducted with modifications to the method described by (Şimşek et al. 2023). Initially, stock solutions of extracts and standards were prepared at 1 mg/ml. For the assay, 100 µl of each extract was diluted to 1.25 ml with 0.2 M phosphate buffer (pH 6.6). Then, 1.25 ml of 1% potassium ferricyanide [K₃Fe(CN)₆] was added, and the mixture was incubated at 50°C for 20 min. After incubation, 1.25 ml of 10% trichloroacetic acid (TCA) and 0.25 ml of 0.1% FeCl₃ were added. The absorbance was measured at 700 nm, and results were expressed in mg TE/g extract using a Trolox calibration curve. The results were compared with Ascorbic acid, BHA, and BHT to assess the antioxidant activity of the samples.

Total phenolic content

The total phenolic content of the extracts was determined using the Folin–Ciocalteu method (Akman et al. 2024). Extract solutions (100 µl) were diluted with 4.5 ml distilled water, followed by the addition of 100 µl Folin–Ciocalteu reagent and 300 µl of 2% Na₂CO₃ solution. The mixture was incubated for 2 h, and absorbance was measured at 760 nm. Results were expressed as mg gallic acid equivalent phenolic substance/g extract (mg GAE/g extract).

Total flavonoid content

To determine the total flavonoid content, the method by Akşit et al. was used (Akşit et al. 2022). Ethanol-prepared stock solutions (100 µl) of extracts and standards were placed in test tubes and adjusted to 4.8 ml with ethanol. Then, 100 µl of 1 M ammonium acetate (NH₄CH₃COO) and 100 µl of 10% aluminium chloride (AlCl₃) were added. The mixture was vortexed and incubated for 45 min at room temperature. Absorbance was measured at 415 nm using a spectrophotometer, and results were expressed as mg quercetin equivalent/g extract (mg QE/g extract).

Antibacterial analysis

In this antibacterial analysis, eight ATCC strains were utilised: five gram-positive (*Listeria monocytogenes* ATCC 51774, *Enterococcus faecium* ATCC 8459, *Clostridium perfringens* ATCC 13124, *Staphylococcus aureus* ATCC 6538, *Bacillus cereus* ATCC 10876) and three gram-negative (*Pseudomonas aeruginosa* ATCC 15442, *Escherichia coli* ATCC 25922, *Salmonella enteritidis* ATCC 13076). The Disc diffusion method (NCCLS 1999) was employed to measure antibacterial activity, with samples dissolved

in 10% DMSO at a concentration of 5 mg/ml. Sterile discs were infused with 20 μ L of the sample and incubated at 37°C for 24 h. Inhibition zones were measured in triplicate. Minimum Inhibitory Concentration (MIC) was determined using a Resazurin-based 96-well plate microdilution method (Akşit et al. 2024). Serial dilutions of the samples (1.95–1000 μ g/ml) were prepared, and bacterial growth was indicated by a colour change from purple to pink after 6 h of incubation at 37°C. The MIC was recorded as the lowest concentration before the colour change.

Statistical analysis

The data obtained in this study were statistically evaluated. A value of $p \leq 0.05$ was accepted as significant in statistical calculations and comparisons. The data were analysed by ANOVA test with 95% confidence intervals and the differences between habitats were determined by S-N-K and Tukey's B in multiple comparisons with SPSS 22 Statistical Software. Correlations between antioxidant activities and phenolic compounds were determined using the Pearson correlation coefficient (r). Differences at $p < 0.05$ were considered significant in the same programme (Elveren and Osma 2022).

Results

In this comprehensive study, the concentrations and antibacterial activities of various antioxidant compounds, including DPPH, TPC, TFC, and FRAP, were analysed in the flowers of 20 different plant species grown across diverse habitats. The results revealed significant variations in the levels of these compounds depending on the habitat from which the samples were collected, as detailed in Table 1.

Upon detailed examination of the data on DPPH free radical scavenging activity, it was observed that the flowers of *A. squamata* L. (24.83 ± 1.25 μ g/ml), collected from the serpentine region, exhibited the highest scavenging activity. This indicates a significant antioxidant potential in this species. The high DPPH scavenging activity suggests that the flowers of *A. squamata* L. possess compounds capable of donating hydrogen atoms or electrons to neutralise free radicals, thereby preventing oxidative stress and potential cellular damage. This finding is particularly noteworthy as it highlights the potential of *A. squamata* L. as a natural source of antioxidants, which could be beneficial in developing therapeutic agents aimed at combating oxidative stress-related diseases.

In terms of total phenolic content, *C. pseudoscammania* C. Koch., also sourced from the serpentine region, demonstrated the highest concentration, measuring 67.92 ± 0.37 . This high phenolic content suggests a robust capacity for free radical neutralisation, contributing to the plant's overall antioxidant efficacy. The elevated levels of phenolics in *C. pseudoscammania* C. Koch. indicate that this plant may have significant health-promoting properties, including anti-inflammatory, anti-carcinogenic, and cardioprotective effects. These properties make it a promising candidate for further research and potential use in nutraceuticals and functional foods.

When analysing the total flavonoid content, the flowers of *T. Heterotomum* (43.48 ± 2.08 mg QE/g extract), collected from the gypsum region, showed the highest amount. Flavonoids are known for their potent antioxidant properties, and this high content underscores the potential health benefits of this species. The substantial flavonoid

Table 1. The in vitro evaluation of TPC, TFC, FRAP, and DPPH' activities of plant flowers.

Habitat	Plant Species	DPPH IC ₅₀ (µg ml ⁻¹)	Total phenolics mg GAE g ⁻¹ Extract	Total flavonoids mg QE g ⁻¹ Extract	Reducing power µg TE g ⁻¹ Extract
Gypsum	<i>Linum flavum</i> L	82.62 ± 1.16	2.70 ± 1.20 ^a	0.99 ± 1.08 ^a	1.75 ± 0.19 ^a
	<i>Paracaryum stenolophum</i>	148.56 ± 2.77	20.54 ± 1.36 ⁱⁱ	13.96 ± 0.63 ^g	19.80 ± 0.69 ^{de}
	<i>Onobrychis nitida</i>	77.84 ± 2.29	26.86 ± 0.85 ^m	19.56 ± 0.73 ^h	16.88 ± 0.49 ^b
	<i>Gypsophila lepidioides</i>	233.1 ± 3.17	10.71 ± 2.21 ^c	7.17 ± 0.74 ^d	16.48 ± 1.92 ^b
	<i>Psephellus recepii</i>	>250	8.51 ± 1.15 ^b	4.82 ± 0.27 ^b	16.40 ± 0.51 ^b
	<i>Tanacetum heterotomum</i>	30.02 ± 0.84	47.17 ± 1.02 ^f	43.48 ± 2.08 ⁱ	23.15 ± 1.02 ^h
	<i>Verbascum alyssifolium</i>	99.46 ± 0.95	17.61 ± 1.45 ^g	11.45 ± 0.39 ^f	18.34 ± 0.93 ^b
	Limestone <i>Teucrium leucophyllum</i>	25.60 ± 2.47	23.98 ± 1.02 ^k	14.12 ± 2.08 ^g	21.50 ± 1.74 ^{fg}
	<i>Stachys sparsipilosa</i>	45.06 ± 1.33	29.26 ± 1.05 ⁿ	6.24 ± 0.69 ^{cd}	19.94 ± 1.29 ^e
	<i>Phlomis oppositiflora</i>	38.26 ± 1.90	19.63 ± 0.98 ^h	8.33 ± 1.94 ^e	19.72 ± 0.91 ^{de}
Serpentine	<i>Pelargonium endlicherianum</i>	13.74 ± 2.75	12.72 ± 1.46 ^d	11.54 ± 1.14 ^f	17.27 ± 0.60 ^b
	<i>Chrysophthalmum montanum</i>	134.67 ± 0.31	14.80 ± 0.89 ^e	6.83 ± 0.23 ^d	16.40 ± 1.00 ^b
	<i>Convulvulus pseudoscammania</i>	24.95 ± 0.94	89.44 ± 0.76 ^t	18.60 ± 0.61 ^h	22.91 ± 0.73 ^h
	<i>Fumana aciphylla</i>	55.51 ± 1.31	21.17 ± 1.34 ^j	5.20 ± 0.67 ^{bc}	18.86 ± 0.49 ^{cd}
	<i>Gladiolus halophilus</i>	>250	15.99 ± 0.29 ^f	8.72 ± 0.73 ^e	16.18 ± 0.88 ^b
	<i>Hypericum thymbrifolium</i>	50.93 ± 2.03	39.39 ± 2.19 ^p	23.65 ± 1.75 ⁱ	21.02 ± 1.00 ^f
	<i>Salvia indica</i>	77.61 ± 1.85	25.31 ± 2.55 ^l	13.94 ± 0.63 ^g	18.53 ± 0.71 ^b
	<i>Artemisia squamata</i>	24.83 ± 1.25	36.65 ± 0.44 ^o	22.79 ± 2.24 ⁱ	18.48 ± 1.21 ^c
	<i>Erysimum pulchellum</i>	142.16 ± 0.72	67.92 ± 0.37 ^s	19.75 ± 1.91 ^h	22.29 ± 0.79 ^{gh}
	<i>Consolida olopetala</i>	112.14 ± 1.77	16.57 ± 1.01 ^b	12.74 ± 0.23 ^{cd}	32.14 ± 1.84 ^b
	<i>Trolox</i>	11.95 ± 0.15	–	–	–
	<i>BHA</i>	8.04 ± 0.69	–	–	338.57 ± 0.31
	<i>BHT</i>	10.70 ± 0.73	–	–	257.80 ± 1.24
	<i>Ascorbic acid</i>	9.91 ± 0.87	–	–	394.17 ± 0.98

content in *T. heterotomum* (Bornm.) Grierson suggests that this plant could be a valuable source of natural antioxidants, with potential applications in pharmaceuticals, cosmetics, and dietary supplements.

Additionally, the FRAP activity was found to be highest in the flowers of *C. Olopetala* (32.14 ± 1.84 mg TE/g extract), another species from the serpentine region. This high FRAP activity indicates a strong ability to reduce ferric ions, further highlighting the antioxidant capacity of this plant. The high FRAP value in *C. olopetala* (Boiss) Hayek suggests that this plant has a significant reducing power, which is indicative of its potential to act as a natural antioxidant. This property is essential for protecting cells from oxidative damage and maintaining overall cellular health. The findings suggest that *C. olopetala* (Boiss) Hayek could be explored further for its potential use in preventing or managing oxidative stress-related conditions.

Following a comprehensive analysis of the data, it was determined that the concentration of antioxidant compounds was significantly higher in plants growing in serpentine and gypsum regions compared to those in other habitats. Statistical evaluations revealed notable differences between habitats, particularly highlighting that plants from serpentine and gypsum areas exhibited distinct antioxidant profiles compared to

those from calcareous soil environments. Further statistical analysis indicated differences in correlation relationships both within individual habitats and across all plant samples (Table 2). In the flowers of the plants studied, significant relationships were observed between antioxidant activities and the phenolic and flavonoid contents, albeit with varying degrees of statistical strength. Specifically, a negative correlation was detected between phenolic content and DPPH activity ($r = -0.32$), as well as between flavonoid content and DPPH activity ($r = -0.46$). While these R-values indicate weak to moderate associations, they support the hypothesis that higher phenolic and flavonoid contents may not directly enhance DPPH radical scavenging activity in certain plant matrices. These findings may reflect the complex interplay of structural and environmental factors influencing the antioxidant efficacy of these compounds. Specifically, in the flowers of the plants studied, a negative correlation was observed between phenolic content and DPPH activity ($r = -0.32$), as well as between flavonoid content and DPPH activity ($r = -0.46$). Conversely, a positive correlation was found between phenolic content and FRAP activity ($r = 0.44$), and a strongly positive correlation was noted between flavonoid content and FRAP activity ($r = 0.46^*$). These results underscore the influence of habitat on the antioxidant compound profiles of plants, suggesting that environmental factors play a crucial role in determining the phytochemical composition and potential health benefits of plant species.

The antibacterial properties of twenty distinct samples were rigorously assessed against a panel of eight pathogenic microorganisms. The detailed outcomes of these assessments are systematically documented in Table 3. Among the twenty samples evaluated, sixteen did not exhibit any significant antibacterial activity against the tested strains. However, four samples, specifically those derived from *S. sparsipilosa*, *T. heterotomum*, *T. leucophyllum*, and *V. alyssifolium*, demonstrated notable antibacterial properties. These samples were effective against *E. faecium* (ATCC 8459) and *S. enteritidis* (ATCC 13076), as detailed in Table 3.

To further elucidate the antibacterial potential of these four active samples, The MIC analysis was conducted. The analysis revealed that a concentration of 1000 µg/ml of *S. sparsipilosa*, *T. heterotomum*, *T. leucophyllum*, and *V. alyssifolium* was sufficient to inhibit the growth of the *E. faecium* strain. Additionally, it was observed that *T. leucophyllum* exhibited inhibitory effects on *S. enteritidis* at a concentration of 31.25 µg/ml, while *S. sparsipilosa* inhibited the same bacterium at a concentration of 62.5 µg/ml (Table 3). The samples in question were found to contain significant quantities of total phenolic and total flavonoid compounds, as indicated in Table 1. It is hypothesised that the antibacterial efficacy of these samples is primarily attributable to their secondary metabolites. Generally, the antimicrobial effects of natural samples are believed to

Table 2. Correlation among TPC, TFC, DPPH, and FRAP of plant flowers grown in different habitats.

DPPH	TPC	-0.32
TPC	TFC	0.63**
TPC	FRAP	0.44
TFC	FRAP	0.46*
DPPH	TFC	-0.46*
DPPH	FRAP	-0.15

** $p \leq 0.01$. * $p \leq 0.05$.

Table 3. The *in vitro* results on the antibacterial activity of plant flowers.

Sample	<i>P.aeruginosa</i>	<i>E.coli</i>	<i>S.enteritidis</i>	<i>B.cereus</i>	<i>L.monocytogns</i>	<i>E.faecium</i>	<i>C.perfringens</i>	<i>S.aureus</i>
					inhibition zone (in mm)			
<i>V. alyssifolium</i>	n.i.	n.i.	8	n.i.	n.i.	9	n.i.	n.i.
<i>T. leucophyllum</i>	n.i.	n.i.	16	n.i.	n.i.	8	n.i.	n.i.
<i>T. heterotomum</i>	n.i.	n.i.	9	n.i.	n.i.	8	n.i.	n.i.
<i>S. sparsipilosa</i>	n.i.	n.i.	14	n.i.	n.i.	9	n.i.	n.i.
*Imipenem	26	30	28	19	n.i.	25	22	21
					MIC (in µg/ml)			
<i>V. alyssifolium</i>			>500			>500		
<i>T. leucophyllum</i>			31.25			>500		
<i>T. heterotomum</i>			>500			>500		
<i>S. sparsipilosa</i>			62.5			>500		

*Note: The inhibition zones are shown in mm *: Positive control (Imipenem), **n.i.: no inhibition *** Other plants not presented in this table did not show any antimicrobial activity against the 8 bacterial species studied.

result from interactions with the bacterial cell wall and cytoplasmic molecules, which are influenced by these secondary metabolites. This hypothesis aligns with the broader understanding that secondary metabolites play a crucial role in the antimicrobial activity of natural products, often through mechanisms involving disruption of bacterial cell walls and interference with intracellular processes.

Discussion

This study investigated the antioxidant and antibacterial properties of twenty plant species from serpentine, gypsum, and limestone regions in Turkey. The primary objective was to correlate habitat-specific conditions with bioactive compound concentrations. Results demonstrated significant variability in TPC, TFC, FRAP, and DPPH activities based on habitat. Plants from serpentine regions exhibited distinct phytochemical profiles compared to those from gypsum and limestone areas, underscoring the impact of environmental factors on bioactive compound biosynthesis. Additionally, antibacterial assays identified certain species with notable antibacterial properties, suggesting their potential as natural antibacterial agents. These findings highlight the importance of habitat conditions in shaping phytochemical composition and biological activities, providing insights for future research and natural product-based therapeutics.

The antioxidant activity of plants, as assessed through various assays such as DPPH free radical scavenging activity, FRAP, TPC, and TFC, exhibits significant variability influenced by the plants' habitats (Lin and Lai 2006). Notably, species originating from serpentine and gypsum regions demonstrate markedly higher antioxidant activity compared to those from limestone areas. This phenomenon aligns with the findings of Çekiç et al., (2021) and Khatri and Chhetri (2020), who observed that plants thriving in stress-prone environments, such as serpentine soils rich in heavy metals, tend to accumulate elevated levels of phenolic and flavonoid compounds as a defensive response (Khatri and Chhetri 2020; Cekic et al. 2021).

The antioxidant potential of plant extracts is intrinsically linked to their phytochemical composition, particularly the presence of phenolics and flavonoids, which are renowned for their capacity to neutralise free radicals and mitigate oxidative stress (Zengin et al. 2015). The findings of this study underscore the profound impact of habitat conditions on the phytochemical profile and bioactivity of plants (Lin and Lai 2006).

Serpentine soils, distinguished by elevated concentrations of heavy metals such as nickel, chromium, and cobalt, coupled with deficient levels of essential nutrients including nitrogen, phosphorus, and potassium, exert significant stress on plant growth. This environmental condition precipitates oxidative damage and induces nutrient deficiencies, thereby impeding plant development (Rajakaruna and Boyd 2014). In response to environmental stressors, plants enhance their antioxidant defense mechanisms by upregulating reactive oxygen species (ROS)-scavenging enzymes such as superoxide dismutase (SOD) and catalase (CAT). These enzymes play a crucial role in detoxifying harmful ROS and preserving cellular integrity (Shafiq et al. 2021; Yang et al. 2022). This stress also induces the biosynthesis of secondary metabolites, including phenolic compounds and alkaloids, which play a dual role in mitigating oxidative stress and enhancing the plants' adaptability to metal-rich, nutrient-deficient environments.

(Proctor 1971; Meindl et al. 2021). Species such as *A. squamata*, *C. pseudoscammania*, and *C. olopetala* demonstrate these adaptations, exhibiting significant antioxidant activities, as evidenced by their superior DPPH, TPC, and FRAP assay values (Xiong et al. 2006; Kazakou et al. 2010; Osmani et al. 2018). These findings are consistent with studies that emphasise the role of metal-binding compounds and antioxidant enzymes in enabling serpentine plants to thrive under challenging conditions (Xiong et al. 2006; Kazakou et al. 2010). Extensive research on the phytochemical diversity of the *Consolida* genus, particularly *C. ambigua* and *C. regalis*, has revealed a rich composition of bioactive metabolites, including diterpenoid alkaloids, flavonoids, and phenolic acids. These compounds not only enhance antioxidative and anti-inflammatory properties but also provide significant antimicrobial and neuroprotective benefits, underscoring the dual ecological and medicinal significance of these species. Among these metabolites, alkaloids such as lycaconitine and ajaconine play a pivotal role in their pharmacological efficacy, offering a mechanistic explanation for their therapeutic potential. Phenolic acids further contribute to their antioxidative and anti-inflammatory activities. Collectively, these traits highlight the ecological resilience and therapeutic value of *Consolida* species, bridging their adaptive survival strategies with their medicinal importance (Yin et al. 2020).

Species of the genus *Artemisia* have been documented to contain phenolic compounds that significantly contribute to their free radical scavenging activities (Singh et al. 2012). The presence of these bioactive compounds is crucial, as they not only confer antioxidant benefits but also play a pivotal role in the plant's defense mechanisms against environmental stressors. The findings concerning *A. squamata* L. (24.83 ± 1.25 µg/ml) are consistent with the broader literature, which indicates that plants from serpentine soils often exhibit enhanced antioxidant properties, attributable to their unique phytochemical profiles (Isaac et al. 2018). Previous research on *A. squamata* has underscored its exceptional antioxidant and enzyme inhibitory properties, primarily attributed to its high concentrations of phenolic and flavonoid compounds. These metabolites are crucial contributors to its radical scavenging capacity and its ability to modulate enzymatic activities, which align with its traditional medicinal uses. The phenolic content not only serves as a potent primary antioxidant but also promotes synergistic interactions with other bioactive compounds, thereby enhancing its overall therapeutic potential. This dual role highlights the ecological and pharmacological importance of *A. squamata* (Zengin et al. 2015).

The total phenolic content of *C. pseudoscammania* C. Koch., measuring 67.92 ± 0.37 mg GAE/g extract, underscores the significant role of phenolic compounds in the antioxidant capacity of plants from serpentine regions. This finding is particularly relevant when considering the ecological context of serpentine soils, which are characterised by high levels of heavy metals and low nutrient availability. Such conditions often lead to the evolution of unique adaptive traits in plants, including enhanced production of secondary metabolites like phenolics, which are known for their antioxidant properties (Cekic et al. 2021). The *Convolvulus* genus is characterised by a notably diverse phytochemical composition, encompassing flavonoids, alkaloids, terpenoids, and phenolic derivatives, all of which contribute to its extensive bioactivities. Notably, species such as *C. arvensis* and *C. prostratus* have exhibited significant antioxidant, anticancer, and anti-inflammatory activities, which are attributed to their abundant phenolic and

flavonoid content. These bioactivities underscore their traditional medicinal applications, including the treatment of ulcers, neurodegenerative disorders, and oxidative stress-related conditions (Salehi et al. 2020).

Similarly, Gypsiferous soils play a crucial role in enhancing plant antioxidant activities by improving nutrient availability and stress response mechanisms. Gypsum, primarily composed of calcium sulphate (CaSO_4), provides essential nutrients such as calcium (Ca) and sulphate (SO_4^{2-}), which are vital for physiological processes like photosynthesis, nutrient metabolism, and oxidative stress mitigation (Kost et al. 2018; Shafiq et al. 2021). By enhancing soil structure, increasing porosity, and improving water infiltration, gypsum facilitates better root development and nutrient absorption, thereby creating optimal conditions for the synthesis of antioxidant enzymes such as superoxide dismutase (SOD) and catalase (CAT) (Shafiq et al. 2021; Morsy et al. 2022). These enzymes effectively scavenge reactive oxygen species (ROS), reduce lipid peroxidation, and stabilise cellular membranes, thereby protecting plants from oxidative damage (Morsy et al. 2022). Additionally, calcium from gypsum modulates the production of secondary metabolites such as phenolics and flavonoids, thereby enhancing the antioxidant capacity of plants. This is consistent with findings in *Tanacetum* species, including *T. heterotomum*, *T. densum* subsp. *eginense*, and *T. alyssifolium*, which exhibit significant antioxidant profiles due to their high phenolic and flavonoid contents (Caniklioğlu et al. 2018; Varol et al. 2023). The high flavonoid content in *T. heterotomum*, particularly in the context of its growth in gypsum-rich regions, aligns with existing literature that underscores the relationship between soil composition and the production of plant secondary metabolites (Caniklioğlu et al. 2018; Varol et al. 2023).

The antibacterial analysis of a diverse set of 20 plant species identified four species – *S. sparsipilosa*, *T. heterotomum*, *T. leucophyllum*, and *V. alyssifolium* – that exhibited significant antibacterial activity. These species demonstrated pronounced efficacy against *E. faecium* and *S. enteritidis*, two clinically important pathogenic bacteria. The selective antibacterial activity observed in these plant species is noteworthy, suggesting that specific secondary metabolites present in these plants may possess potent antimicrobial properties. The observed antibacterial activity, especially the low MIC values of *T. leucophyllum* against *S. enteritidis*, supports the hypothesis that phenolic compounds and flavonoids play a significant role in plant defense mechanisms against microbial pathogens by forming complexes with bacterial cell components, leading to the disruption of the cell wall and subsequent inhibition of growth (Barroqueiro et al. 2016; Górniak et al. 2019). This hypothesis aligns with the observed antibacterial efficacy of *T. leucophyllum* against *S. enteritidis*, reinforcing the theory that these compounds play a crucial role in plant defense (Barroqueiro et al. 2016; Yang et al. 2016; Górniak et al. 2019). The significant antibacterial activity observed in plants from serpentine and gypsum regions can be attributed to their unique phytochemical profiles.

Conclusion

This study systematically examines the production of secondary metabolites, specifically phenolic compounds and flavonoids, in the flowers of plant species to serpentine, gypsum, and limestone ecosystems. It emphasises their crucial role in enhancing antioxidant capacity and antibacterial activity within these unique ecosystems. By focusing

exclusively on floral components, the research highlights the biochemical adaptation mechanisms to environmental stresses, particularly through elevated levels of phenolic and flavonoid compounds. The findings revealed significant variations in the concentration of antioxidant compounds across different habitats, with serpentine and gypsum soils yielding higher amounts of these bioactive compounds. Notably, species from serpentine habitats demonstrated superior DPPH radical scavenging activity and FRAP values, underscoring the adaptive biochemical responses of plants to the harsh conditions of these environments. Furthermore, the study established a correlation between the phenolic and flavonoid content of the plants and their antioxidant activities, suggesting that these secondary metabolites play a crucial role in the plant's defense mechanisms under abiotic stress. Furthermore, the antibacterial assays identified a subset of plant extracts exhibiting notable inhibitory effects against *E. faecium* and *S. enteritidis*, underscoring the potential of these species as sources of novel antimicrobial agents. The observed antibacterial activity is likely attributable to the high phenolic and flavonoid content, supporting the hypothesis that secondary metabolites contribute to the antimicrobial properties of these plants.

Overall, this research highlights the critical role of habitat-specific factors in shaping the biosynthesis of secondary metabolites in plants and their subsequent biological activities. The distinct phytochemical profiles observed across different habitats underscore the adaptive strategies employed by plants to thrive in their environments. These findings suggest that endemic species could serve as valuable sources of natural antioxidants and antimicrobials. Future research should aim to thoroughly characterise the bioactive compounds responsible for these activities and investigate their potential applications in pharmaceuticals and other industries. This study contributes to the expanding body of knowledge on plant biodiversity and its implications for bioprospecting in regions with unique edaphic conditions.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

Data will be made available on request.

Credit authorship contribution statement

Samed ŞİMŞEK contributed to the investigation and methodology, extraction procedure, antioxidant analysis, and writing the original draft. Zeynep AKŞİT was involved in the methodology, antibacterial analysis, and writing – review & editing. Tuğçe VAROL, Etem OSMA, and Mürşide ELVEREN contributed to the investigation, and writing – review & editing.

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