

PHYTOCHEMICAL PROFILE, ANTIOXIDANT POTENTIAL, AND ANTIMICROBIAL PROPERTIES OF *HEDYSARUM VARIUM* WILLD.

HEDYSARUM VARIUM WILLD. BİTKİSİNİN FENOLİK PROFİLİNİN, ANTİOKSİDAN VE ANTİMİKROBİYAL ÖZELLİKLERİNİN DEĞERLENDİRİLMESİ

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ABSTRACT

Objective: This study aimed to determine the phytochemical profile of *Hedysarum varium*, on which there has been limited research, and to evaluate its antioxidant capacity and antimicrobial effect.

Material and Method: Total phenolic content of extracts obtained from plant material (aerial part and root) using 70% ethanol was determined by the Folin-Ciocalteu method. The antioxidant potential of the extracts was evaluated by measuring their DPPH free radical scavenging effects and total antioxidant capacity. Qualitative and quantitative analysis of the extracts was performed using High Performance Liquid Chromatography (HPLC). A calibration curve was created using the external standard method for rutin quantification. For antibacterial and antifungal effects, the Minimum Inhibitory Concentration (MIC) value was determined against *S. aureus*, MR *S. aureus*, *E. coli*, *K. pneumoniae*, *C. albicans*, and *C. glabrata* strains using the broth microdilution (BMD) method.

Result and Discussion: As a result of the study, HPLC analysis detected the presence of rutin in the extract of the aerial parts, and its amount was determined to be 0.85 ± 0.01 mg/100 mg dry plant. The extract of the aerial parts was found to have a higher total phenolic content compared to the root extract. The antioxidant capacities of the extracts were found to be parallel to their phenolic content. In antimicrobial activity tests, MIC values were determined to be in the range of 32–256 µg/ml. The extracts were observed to be equal to or less effective than the reference sulfisoxazole against bacterial strains and less effective than the reference ketoconazole against fungal strains.

Keywords: Antibacterial, antifungal, antioxidant, *Hedysarum varium*, rutin, HPLC

ÖZ

Amaç: Bu çalışmada, üzerinde sınırlı çalışma bulunan *Hedysarum varium*'un fitokimyasal profilinin belirlenmesi, antioksidan kapasitesinin ve antimikrobiyal etkisinin değerlendirilmesi amaçlanmıştır.

Gereç ve Yöntem: Bitkisel materyalden (topraküstü ve kök) %70 etanol kullanılarak elde edilen ekstrelerin, toplam fenol miktar tayini Folin-Ciocalteu yöntemiyle yapılmıştır. Ekstrelerin antioksidan potansiyeli, DPPH serbest radikal süpürücü etkileri ile total antioksidan kapasiteleri ölçülerek değerlendirilmiştir. Ekstrelerin kalitatif ve kantitatif analizi Yüksek Performanslı Sıvı

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Kromatografisi (YPSK) ile gerçekleştirilmiştir. Rutin miktar tayini için dış standart yöntemiyle kalibrasyon eğrisi oluşturulmuştur. Antibakteriyel ve antifungal etki için broth mikrodilüsyon (BMD) yöntemiyle S. aureus, MR S. aureus, E. coli, K. pneumoniae, C. albicans ve C. glabrata suşlarına karşı Minimum İnhibitör Konsantrasyon (MIC) değeri olarak belirlenmiştir.

Sonuç ve Tartışma: Çalışma sonucunda, YPSK analizi ile topraküstü ekstrede rutin varlığı tespit edilmiş ve miktarı 0.85 ± 0.01 mg/100 mg kuru bitki olarak belirlenmiştir. Topraküstü kısım ekstresinin, kök ekstresine kıyasla daha yüksek toplam fenolik içeriğe sahip olduğu bulunmuştur. Ekstrelerin antioksidan kapasitelerinin, fenolik içeriklerine paralel olduğu belirlenmiştir. Antimikrobiyal aktivite testlerinde MIC değerlerinin 32–256 µg/ml aralığında olduğu belirlenmiştir. Ekstrelerin, bakteri suşlarına karşı referans sülfizoksazola kıyasla eşit veya daha az; mantar suşlarına karşı ise referans ketokonazoldan daha az etkili olduğu gözlemlenmiştir.

Anahtar Kelimeler: Antibakteriyel, antifungal, antioksidan, *Hedysarum varium*, rutin, YPSK

INTRODUCTION

Hedysarum varium Willd is classified within the family Fabaceae which is rich in economically valuable, endemic species and contains numerous genera with pharmacologically valuable components [1,2]. Fabaceae family is particularly distinguished by its numerous genera that contain compounds with pharmacological properties, making them valuable for both traditional and modern medicine. The genus *Hedysarum*, commonly referred to in local terms as “batalak,” comprises approximately 100 species, each exhibiting unique adaptations. These species are naturally distributed across a wide geographical range, spanning continents such as Europe, North Africa, Asia, and North America, indicating a significant ecological and evolutionary versatility [3,4,5]. Within the broader spectrum of the *Hedysarum* genus, which includes 154 species worldwide, the majority are perennial herbaceous plants, characterized by their ability to live for multiple years, while a minority exist as semi-shrub forms. One of the most remarkable features of *Hedysarum* species is their exceptional range of ecological tolerance. These plants thrive in various habitats, demonstrating adaptability from the formidable altitudes of high mountains to the harsh environments of polar tundras, as well as in rocky grasslands, scrublands, arid deserts, and the biodiverse coastal ecosystems. This broad habitat range demonstrates their resilience and their ability to occupy ecological niches that are often extreme or challenging [4,6].

In Turkey, the region is home to a remarkable diversity of 21 *Hedysarum* species, with 11 of these endemic, meaning they are naturally found only in this specific geographical area. This noteworthy level of endemism results in an endemism rate of approximately 52.3% for the genus within Turkey, highlighting the ecological importance and biodiversity of the region. This rich natural heritage not only enhances Turkey's landscape but also emphasizes the potential for studying and utilizing these species for ecological research and pharmaceutical purposes [3,5,7].

The genus *Hedysarum* encompasses a variety of species that are utilized by local populations across different regions, each known for its unique properties and benefits. For instance, *Hedysarum alpinum* is noteworthy for its edible roots, which are not only rich in vitamin C but also play a role in traditional remedies for oral lesions. The nutrient content of these roots makes them an important food source in certain communities. Other species such as *Hedysarum polybotrys*, *H. limprichtii*, *H. vicioideri*, and *H. smithianum* have significant roles in traditional Chinese medicine. These species are often revered for their tonic properties, believed to enhance overall vitality and health. The use of these plants highlights the deep relationship between local cultures and their natural environment, as well as the value placed on herbal remedies in traditional healing practices [3].

Hedysarum species are a rich source of biologically important secondary metabolites such as flavonoids, coumarins, triterpenoids, saponins, lignoids, sterols, nitrogen-containing compounds, carbohydrates, and fatty acids and related components [1,3,8,9]. High levels of protein and condensed tannins, with phenolic compounds belonging to the flavonoid class (such as kaempferol, quercetin, genistein, formononetin, and afmosin), have been detected in the aerial parts of plants in the genus [10]. Current phytochemical studies have shown that phenolic compounds such as liquiritigenin, quercetin, formononetin, and genistein are predominantly found in the roots of *H. polybotrys*. In contrast, *H. alpinum* and *H. flavescens* are reported to be particularly rich in flavonoids and phenolic acids, including mangiferin, hyperoside, rutin, quercetin-3-*O*-rhamnoside, avicularin, polystachoside,

gallic acid, and chlorogenic acid. Additionally, it has been reported that mangiferin, a xanthone-derived compound, is present in high amounts particularly in *H. caucasicum*, *H. grandiflorum*, and *H. daghestanicum* species [1,11]. These components have been associated with antioxidant, antitumor, immunomodulatory, antidiabetic, antiaging, and antihypertensive effects [1,3]. *In vitro* studies, particularly those using root extracts, have reported significant antiproliferative activity against human hepatocellular carcinoma and gastric cancer cells, indicating potential applications in cancer treatment [1].

Scientific studies on the species *H. varium* are limited. In a study conducted to identify new rutin sources and understand the differences between analytical techniques, rutin was detected for the first time by HPLC in the flowers of *H. varium*, and its content was found to be 5.18% [12,13]. For *H. varium*, one of the species examined in the study by Zengin et al., ethyl acetate, methanol, and water extracts showed: low to medium phenolic/flavonoid content; medium antioxidant capacity tests; a limited effect on metal chelation; and low potency compared with references in AChE/BChE, α -amylase, tyrosinase, and α -glucosidase inhibition. The corresponding IC_{50} and EC_{50} values were higher than those of galantamine, kojic acid, acarbose, and trolox; activity varied depending on extract polarity, with the antioxidant response increasing as the phenolic load increased [14].

The aim of this study was to determine the phytochemical profile of aqueous ethanol extracts prepared from the aerial and root parts of the *Hedysarum varium* and to evaluate their antioxidant and antimicrobial activities. High-performance liquid chromatography (HPLC) was used to identify the active compound.

MATERIAL AND METHOD

Plant Material

H. varium was collected from Yaşamkent, Çankaya, Ankara (39°50'38.0"N 32°39'01.0"E) on 13 June 2025. The herbarium specimen was pressed and dried, identified by Prof. Dr. A. Mine Gençler ÖZKAN, and then registered in the Herbarium of the Faculty of Pharmacy, Ankara University, under the number AEF 3129.

Extraction

The aerial parts and roots of *H. varium* were ground. The powdered plant material was macerated at room temperature for 24 hours using 70% ethanol at a ratio of 1 g plant/10 ml solvent. After maceration, the extracts were subjected to 1 hour of ultrasonic treatment and filtered through filter paper. The extraction process was repeated 7 times for each extract. The filtrates were combined and evaporated to dryness at 40°C. The obtained extracts were stored at 4°C until use.

Total Phenol Content Determination

The total phenol content of the extracts was determined using a modified Folin Ciocalteu method [15]. 10% (w/w) Folin Ciocalteu reagent and 5% (w/v) sodium carbonate solution were added to the samples, respectively, and incubated. The total phenolic content of each extract was calculated as mg gallic acid equivalent/g dry extract (mg GAE/g dry extract) according to the calibration curve obtained with the reference substance gallic acid. Each analysis was performed in triplicate.

High-Performance Liquid Chromatography (HPLC) Analyses

Qualitative analysis of total extracts prepared from the aerial parts and roots of plant material using 70% ethanol was performed using an HPLC. Stock solutions of the total extracts were prepared at a concentration of 15 mg/ml, and each was separately filtered through 0.45 μ m filters to prepare the HPLC samples of the extracts. The conditions of the qualitative analysis performed by HPLC are shown in Table 1.

Table 1. HPLC Analysis Conditions

HPLC Instrument	Agilent 1260 Infinity Series
Column Specifications	ACE 5 C18 (250 mm×4.6 mm, 5 µm)
Detector Type	Diode Array Detector
Wavelength	210 nm
Flow Rate	1 ml/min
Injection Volume	10 µL
Solvent System	0.2% <i>o</i> -phosphoric acid in water-acetonitrile

The gradient solvent systems and column temperatures used in the qualitative analysis performed with HPLC are listed in Table 2.

Table 2. The solvent system used in the analysis

Column Temperature	Time	Acetonitrile (%)	2% <i>o</i>-Posphoric Acid in Water
40°C	0	5	95
	50.00	20	80
	50.01	90	10
	55.00	90	10

Preparation of Standard Series

The external standard method was used to determine the amount of the substance detected in the extract during qualitative analysis. For this purpose, a series of solutions consisting of concentrations within the linearity range of the standard were prepared and analyzed. Three analyses were performed for each concentration, and the calibration equation was created using the average of the peak area under the curve for the substance. The area of the peak identified as belonging to the substance in the extract was substituted into this equation to calculate the amount of the substance. To determine the amount of the compound, analyses were performed at concentrations of 0.01- 1 mg/ml for the rutin.

Determination of the Detection and Quantification Limits

The detection limit (LOD) and quantification limit (LOQ) values of the analyzed substance were calculated considering the signal-to-noise ratio, and six analyses were performed on samples prepared at this concentration.

Determination of Antioxidant Capacity

Free Radical (DPPH) Scavenging Capacity Assay

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity method was modified to determine the antioxidant capacity of the extracts [16]. In the study, different concentrations (25-1000 µg/ml) of each extract were added to the DPPH solution and incubated at room temperature in the dark for 30 minutes. Subsequently, absorbance measurements were performed at a wavelength of 517 nm using a microplate reader. Ascorbic acid was used as reference substance. The percentage inhibition value of the extract solutions at different concentrations in scavenging the DPPH free radical was determined using the following equation.

$$\% \text{ inhibition} = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}}) / \text{Absorbance}_{\text{Control}}] \times 100$$

Calibration graphs were drawn for each plant extract using the % inhibition values at different concentrations, and the half-maximal inhibition concentration (IC₅₀) values were determined.

Determination of Total Antioxidant Capacity

The phosphomolybdenum method was modified [17]. Different concentrations (10-1000 µg/ml) of the extracts and ascorbic acid were treated with a reagent solution prepared from a mixture of sodium phosphate, ammonium molybdate, and sulfuric acid. After incubation of the mixture in an oven at 95°C, the absorbances of the microplates were measured spectrophotometrically at 765 nm. Each experiment was performed in triplicate. The total antioxidant capacity of the extracts was calculated as mg ascorbic acid equivalent/g dry extract (mg AAE/g dry extract) according to the calibration curve obtained with the reference substance ascorbic acid.

Antibacterial/Antifungal Susceptibility Assay

The antimicrobial potential of the investigated extracts was examined against a panel of microorganisms, including Gram-positive strains (*Staphylococcus aureus* ATCC 25923 and methicillin-resistant *S. aureus* ATCC 15193), Gram-negative strains (*Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 4352), as well as yeasts (*Candida albicans* ATCC 10231 and *Candida glabrata* ATCC 2950). These organisms originated from the reference strain collection of the Medical Microbiology Laboratory, Faculty of Medicine, Yüksek İhtisas University (Ankara, Türkiye). Prior to experimentation, all isolates were routinely maintained on blood agar (OXOID, Italy) and incubated aerobically at 37 °C for 24–48 h to ensure viability.

Antibacterial and antifungal susceptibility profiles were determined using the broth microdilution approach, following CLSI performance standards [18]. A series of ten two-fold dilutions (0.5–256 µg/ml) of each plant extract was prepared. Microbial inocula were standardized to approximately $1.5\text{--}2.0 \times 10^5$ CFU/ml, and 100 µl of each suspension was dispensed into wells containing the designated concentration of extract. Appropriate sterility and growth controls were incorporated into each assay plate. After incubation at 35 °C for 24 h, microbial proliferation was evaluated visually, and MIC values were established as the lowest concentration that inhibited visible growth. Sulfisoxazole and ketoconazole served as reference drugs for antibacterial and antifungal comparisons, respectively.

RESULT AND DISCUSSION

The prepared dry extracts were weighed, and the yields of the aerial and root extracts were 16.86% and 9.92%, respectively. The aerial part extract was analyzed, revealing the presence of rutin, quantified at a wavelength of 210 nm. The HPLC chromatogram corresponding to the extract obtained from the plant's aerial parts is displayed in Figure 1.

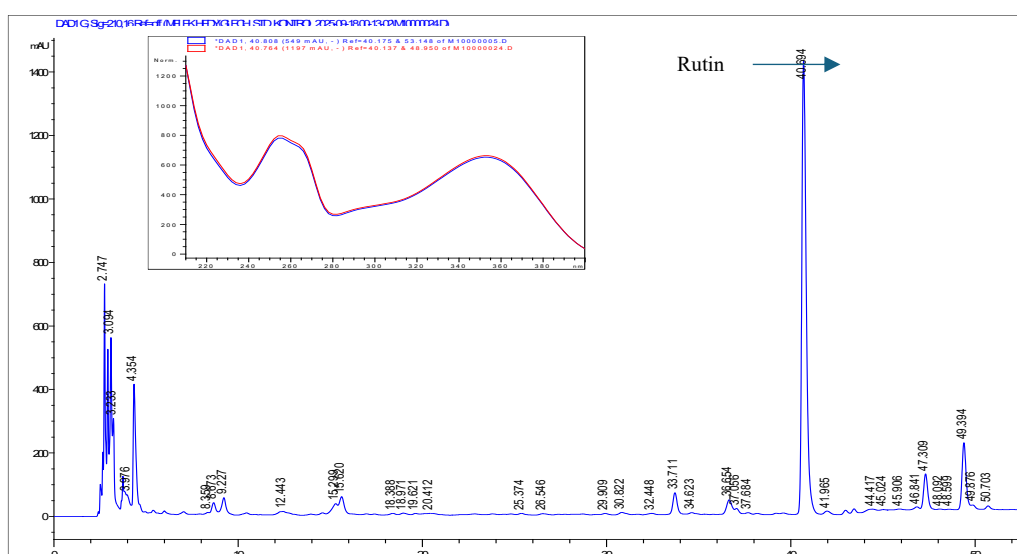


Figure 1. Chromatogram of the extract prepared from the aerial parts of *H. varium* with 70% ethanol at a wavelength of 210 nm (Overlapped UV spectrum of rutin)

The HPLC chromatogram at the wavelength used for quantification and UV spectrum of rutin are shown in Figure 2.

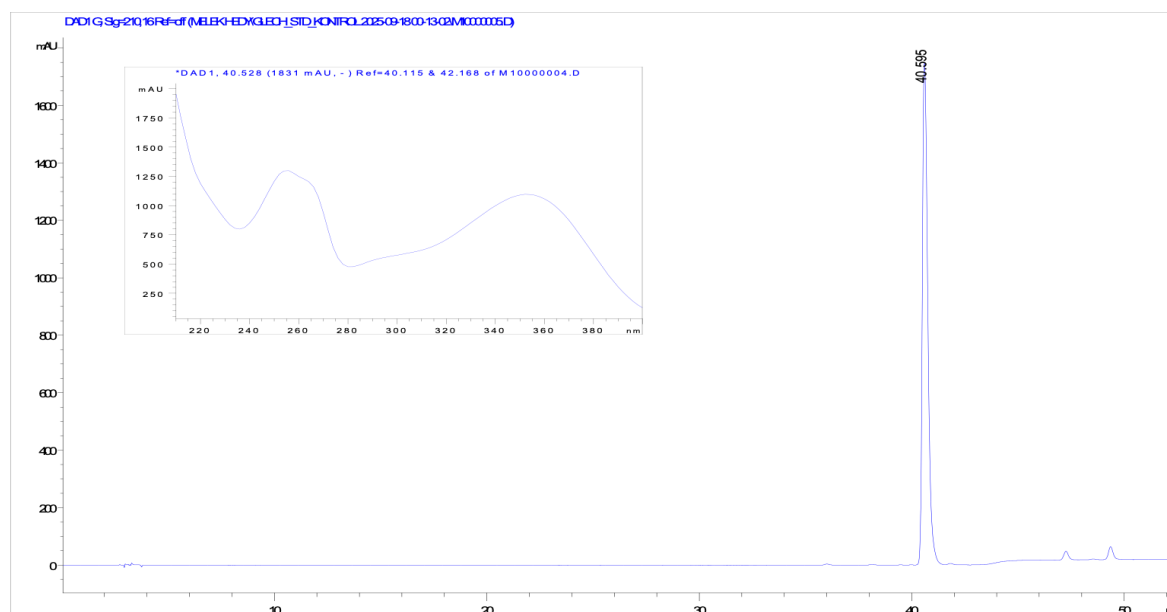


Figure 2. Chromatogram and UV spectrum of the rutin standard at 210 nm wavelength

The calibration equation for the standard substance was derived from area-under-the-curve values and concentration data obtained from the chromatograms of the standard series. The calibration equation for rutin in the aerial extract of *H. varium* is $y = 31893x + 165.08$, with $R^2 = 0.9994$. The mean rutin content is 0.85 mg per 100 mg of plant material (SD = 0.0144). The LOD, calculated for the instrument and method used for quantitative analysis, is 0.43 $\mu\text{g/ml}$, and the LOQ value is 1.44 $\mu\text{g/ml}$.

HPLC analysis confirmed the presence of rutin, a flavonoid recognized for its pharmacological importance, in the extract of the aerial parts and quantified its content as 0.85 mg per 100 mg of plant material. This value differs markedly from the high value of 5.18% reported by Toker et al. for the same species [13]. The significant discrepancy can be attributed to Toker et al. focusing exclusively on rutin-enriched tissues, such as flowers, while this investigation encompasses all aerial components, including stems and leaves.

Table 3 provides a summary of the biological activity results for the *H. varium* extracts, including their total phenolic content (Folin-Ciocalteu), DPPH radical scavenging capacity, and total antioxidant capacity (phosphomolybdenum method).

In the analyses conducted, it was determined that the aerial parts of the *H. varium* plant (105.77 mg GAE/g dry extract) were approximately 2.5 times richer in phenolic compounds than the root parts (44.48 mg GAE/g dry extract). This phytochemical superiority is also reflected in biological activity. In the DPPH radical-scavenging capacity test, ascorbic acid, used as a positive control, exhibited very strong antioxidant potential, as expected, with an IC_{50} of 0.0097. When *H. varium* extracts were examined, the aerial parts extract showed lower activity than ascorbic acid and had an IC_{50} value of 0.12 mg/ml, but it demonstrated a remarkable radical-scavenging capacity for a total extract. The root extract exhibited weak activity, with an IC_{50} of 1.13 mg/ml. The aerial parts extract also exhibited significantly higher total antioxidant capacity than the root extract, with a value of 62.45 mg AAE/g. The total phenolic content (TPC) of 105.77 mg GAE/g dry extract obtained in this study was even higher than the highest TPC value reported by Zengin et al. for solvent extracts of the same plant with different polarity (MeOH extract: 45.11 mg GAE/g dry extract). Similarly, our DPPH IC_{50} value of 0.12 mg/ml was lower than those of the most active MeOH extract (0.30 mg/ml) and the positive control Trolox (0.31 mg/ml).

in the study by Zengin et al. [14]. This difference may be attributed to variation in factors such as the collection locality, the collection season, or the 70% ethanol-extraction technique used in our study.

Table 3. Total phenolic content, DPPH radical scavenging activity, and total antioxidant capacity of *H. varium* aerial part and root extracts

Plant Extracts/ Positive Control	Total Phenolic Compound Content mg gallic acid equivalent / g dry extract Mean±SD	DPPH Radical Scavenging Activity IC ₅₀ (mg/ml) Mean±SD	Total Antioxidant Capacity mg ascorbic acid equivalent /g dry extract Mean±SD
<i>H. varium</i> (Aerial part)	105.7675 ± 3.9846	0.1234 ± 0.0090	62.4469 ± 2.7254
<i>H. varium</i> (Root)	44.4784 ± 4.1304	1.1317 ± 0.0185	46.3415 ± 3.8150
Ascorbic Acid (Positive control)	-	0.0097 ± 0.0018	-

The antimicrobial efficacy of the extracts was determined using the broth microdilution assay. The MIC values obtained for Gram-positive, Gram-negative, and fungal strains, including *S. aureus* ATCC 25923, MRSA ATCC 15193, *E. coli* ATCC 25922, *K. pneumoniae* ATCC 4352, *C. albicans* ATCC 10231, and *C. glabrata* ATCC 2950, are summarized in Table 4. Overall, extract concentrations that inhibited microbial growth ranged from 32 to 256 µg/ml. Specifically, MIC values for *H. varium* root extract ranged from 64 to 256 µg/ml, whereas those for the aerial parts ranged from 32 to 128 µg/ml. When evaluated relative to standard antimicrobial agents, the extracts exhibited comparable or variable inhibitory performance against *S. aureus* ATCC 25923, MRSA ATCC 15193, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 4352. In contrast, both extracts demonstrated weaker antifungal activity against *C. albicans* ATCC 10231 and *C. glabrata* ATCC 2950 when compared with reference drugs (Table 4).

Table 4. MIC values (µg/ml) of the *H. varium* aerial part and root extracts

Plant Extracts/ Positive Controls	<i>Staphylococcus aureus</i> ATCC 25923	Methicillin-resistant <i>S. aureus</i> (MRSA) 15193	<i>Escherichia coli</i> ATCC 25922	<i>Klebsiella pneumoniae</i> ATCC 4352	<i>C. albicans</i> (ATCC 10231)	<i>Candida glabrata</i> ATCC 2950
	MIC (µg/ml)					
<i>H. varium</i> (Aerial part)	128	32	64	64	64	64
<i>H. varium</i> (Root)	64	256	64	128	64	64
Sulfisoxazole	128	128	64	64	-	-
Ketoconazole	-	-	-	-	32	32

Research on *Hedysarum* species has demonstrated a range of biological activities associated with these plants. Scientific investigations have established that these species warrant further exploration due to their notable antioxidant and antimicrobial properties. One particular study examined extracts from *H. aucheri*, prepared using *n*-hexane, dichloromethane, butanol, methanol, and water, focusing on their phytochemical content and biological properties. The analysis determined the levels of fatty acids, total flavonoids, and phenolics, and assessed the antioxidant, cytotoxic (potentially effective against cancer cells), and antimicrobial (against bacteria and fungi) activities of these extracts. The results revealed that the methanol extract had the highest concentration of total phenolics, while the butanol extract showed the strongest antioxidant capacity. Interestingly, the hexane extract exhibited the most significant

antimicrobial activity. Furthermore, gas chromatography-mass spectrometry (GC-MS) identified 24 fatty acids, with α -linolenic acid constituting 32.37% and linoleic acid accounting for 9.16% of the total composition. The hexane extract showed notable inhibitory effects against *Salmonella* spp., *E. coli*, and *Candida albicans*. Furthermore, the dichloromethane extract displayed strong antiproliferative effects on specific cancer cell lines without harming healthy cells [19].

The antioxidant, antimicrobial, cytotoxic, and enzyme-inhibitory activities of water (HNW) and methanol (HNM) extracts prepared from *H. nitidum* (the aerial parts) were also evaluated previously and their phytochemical profiles were analyzed by GC-MS and HPLC. Caffeic acid, 2-hydroxycinnamic acid, and *p*-hydroxybenzoic acid, were detected by HPLC; n-octanal, hexafarnesyl acetone, humulene epoxy-II, α -terpineol, and eicosane were detected by GC-MS. Both extracts exhibited potent antioxidant and enzyme inhibitory properties. HNM showed selective antibacterial activity against Gram-positive bacteria; neither extract induced cytotoxicity in A549 human lung cancer cells. The findings indicate the potential therapeutic use of *H. nitidum* [1].

In another study, LC-MS/MS analysis of *H. candidissimum* extracts (ethyl acetate, methanol, water) revealed the presence of a total of 22 different phytochemical compounds in the extracts, primarily phenolic acids, flavonoids, and flavonoid glycosides. The methanol extract, which had a high total phenolic and flavonoid contents, stood out for its DPPH radical scavenging and iron reduction capacities, exhibiting the highest antioxidant activity. Additionally, the methanol extract showed the strongest inhibitory effect against acetylcholinesterase (AChE; $IC_{50} = 93.26 \mu\text{g/ml}$) and α -glucosidase (AG; $IC_{50} = 28.57 \mu\text{g/ml}$), *in vitro*. These results were supported by *in silico* analyses, which confirmed that the inhibitory activities originate from the main phenolic components. The strongest cytotoxic activity was observed in the ethyl acetate extract, which inhibited the cancer cell lines such as MDA-MB-453 and HT-29 with IC_{50} values of $95.36 \mu\text{g/ml}$ and $63.03 \mu\text{g/ml}$. Both extracts have been reported to exhibit significant apoptosis-inducing and cell migration-inhibiting properties on HT-29 cells [3].

Furthermore, the chemical composition and biological effects of hydroalcoholic extracts obtained from *in vitro* root cultures of *H. neglectum* transformed by *Agrobacterium rhizogenes* were evaluated. Extraction conditions were varied in terms of temperature (30, 50, and 70 °C), alcohol percentage (30%, 50%, and 70%), and duration (2, 4, 5, and 6 hours); the extracts were detected by HPLC, TLC, and GC-MS, and the presence of quercetin-rhamnopyranoside, quercetin, gallic acid, and mangiferin was detected. Antioxidant and antimicrobial activities were observed in all samples; the highest antioxidant activity was 0.6410 mg/g in the No. 4 *H. neglectum* extract prepared under optimal conditions (50 °C, 50% alcohol, and 4-hour extraction time). This extract was also reported to exhibit the strongest antagonistic effect. The findings suggested a potential neuroprotective effect, and it was stated that these compounds would be isolated and evaluated for functional nutrition [20].

In conclusion, this study provides compelling quantitative evidence that the aerial parts of *H. varium* exhibit significant biological activity, which is strongly associated with their remarkable phenolic content, measured by 105.77 mg GAE/g dry extract. These findings are particularly noteworthy, as the plant exhibited a low minimum inhibitory concentration (MIC) of 32 $\mu\text{g/ml}$ against the methicillin-resistant *Staphylococcus aureus* (MRSA 15193) strain. The selective and pronounced efficacy of *H. varium* against MR *S. aureus* underscores its pharmacological promise, suggesting that it could serve as a valuable resource in combating antibiotic-resistant infections. This clearly demonstrates the essential need for further phytochemical investigations, particularly bioactivity-guided isolation, to effectively identify and extract the specific bioactive compounds responsible for these significant effects.

AUTHOR CONTRIBUTIONS

Concept: Ö.B.A.; Design: M.K., Ü.Ç., Ö.B.A.; Control: Ö.B.A.; Sources: M.K., Ü.Ç.; Materials: M.K., Ü.Ç., Ö.B.A.; Data Collection and/or Processing: M.K., Ü.Ç., Ö.B.A.; Analysis and/or Interpretation: M.K., Ü.Ç., Ö.B.A.; Literature Review: M.K.; Manuscript Writing: M.K., Ü.Ç.; Critical Review: Ö.B.A.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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