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# Antioxidant activity and phenolic–flavonoid content of *Phlomis pungens* var. *pungens* collected in Erzincan (Türkiye)

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## ABSTRACT

**Objective:** In this study, the antioxidant capacity of the methanol:water (7:3, v/v) extract obtained from the aerial parts of Phlomis pungens var. pungens (PP), native to Erzincan province, Turkey, was evaluated.

**Methods:** The antioxidant activity of the herbal extract was investigated by different spectrophotometric methods; 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•+) radical scavenging tests, ferric reducing antioxidant power (FRAP), total phenolic content (TPC) and total flavonoid content (TFC) analyses were carried out.

**Results:** According to the obtained data, the DPPH• and ABTS•+ free radical scavenging capacity of the PP extract was determined to be low compared to synthetic antioxidants ($DPPHIC_{50}$: 99.02 µg/mL, ABTS IC50: 36.04 µg/mL). The 82.45 mg TE/g extract value obtained as a result of the ferric reducing antioxidant power (FRAP) analysis indicates that the reducing capacity of PP is moderate. Total phenolic content was measured as 49.23 mg GAE/g extract, and total flavonoid content was measured as 24.57 mg QE/g extract. These values indicate that the extract has a certain level of antioxidant potential.

**Conclusion:** The findings suggest that PP may be considered a potential natural antioxidant source due to its phenolic and flavonoid compounds, although its activity was observed to be moderate. Furthermore, when compared with the limited number of studies in literature, the results are generally consistent; however, some differences were observed due to factors such as the extraction methods used, solvent ratios, and the environmental conditions in which the plant was collected. Overall, this study contributes valuable data to the current understanding of the biological potential of PP and supplements the limited existing literature on this species.

**Keywords:** *Phlomis pungens, ABTS, DPPH, FRAP, TPC, TFC*

## INTRODUCTION

Throughout history, humans have encountered many illnesses and struggled to eliminate them using various methods. Among the many ways employed in the fight against illnesses, medicinal plants are used to cure a variety of disorders.[1] These medicinal plants have been used in traditional medicine worldwide and are potential reservoirs for new drugs. Common diseases such as Alzheimer's, Parkinson's, inflammatory diseases, lipid peroxidation, DNA damage, celiac disease, cardiovascular diseases, protein oxidation and diabetes are mostly due to the production of free radicals.[2]

Free radicals are inorganic atoms or molecules with unpaired electrons in their outer layers and have high chemical activity. These radicals, which can be formed as a result of enzymatic reactions, auto-oxidation processes and environmental factors (UV rays, air pollution, cigarette smoke, etc.), interact with biomolecules (DNA, lipids, proteins) and cause oxidative stress.[3] Free radicals, which cause damage at the cellular level, also play a role in some physiological processes by taking part in biosignal transmission and electron transfer processes. Uncontrolled oxidative stress can be effective in the development of many diseases such as cancer.[4]

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Organisms have antioxidant defense systems that balance these harmful effects. Antioxidants help neutralize free radicals and help maintain cellular integrity.[5,6] These defense systems, which can be of endogenous or exogenous origin, consist of substances taken in the diet such as vitamins and minerals or molecules produced naturally in the body.[7] Plants stand out as powerful natural antioxidant sources through the phenolic compounds they contain. The hydroxyl groups in the structure of these compounds play a decisive role in antioxidant activity with their capacity to neutralize reactive oxygen species.[3,8,9] For this purpose, species belonging to the Lamiaceae family attract attention due to their rich phytochemical components and pharmacological activities.

The *Lamiaceae* (Ballıbabagiller) family has an important place in the Turkish flora due to its wide distribution and economic value. Phlomis, one of the notable genera of this family, represents a rich group in terms of chemical compound diversity with approximately 100 species.[10,11] Phlomis species contain many biologically active compounds such as iridoids, flavonoids, phenylpropanoids, monoterpenes, lignans and alkaloids, and these components show various pharmacological activities such as antioxidant, anti-inflammatory, antimicrobial and immunosuppressive effects.[12,13] In traditional use, these species are used for stimulant, tonic, diuretic and wound healing purposes, and also attract attention with their antioxidant and immune regulating effects supported by scientific studies.[14]

*Phlomis pungens var. pungens* (PP), known by its Turkish name as silvanok, is a perennial plant belonging to the *Lamiaceae* family, which is widespread in arid and rocky regions of Turkey and is rich in essential oils and phenolic compounds.[14,15] It exhibits wound healing, anti-inflammatory, antibacterial and antifungal effects thanks to the *α*-pinene, germacrene D, flavonoids, iridoids and phenylpropanoid glycosides in its structure.[12,16] In addition, the immunosuppressive and antioxidant properties of these components contribute to the plant's broad biological activity profile. PP has the potential to be used as a natural flavoring and preservative in the food, cosmetics and agricultural sectors thanks to the aromatic compounds it contains.[11,17]

Studies on the antioxidant properties of Phlomis pungens subspecies are quite limited, and only one comprehensive study on the PP species exists in the literature.[10–12,15–19] However, studies on the related species *Phlomis armeniaca*, *Phlomis russeliana Phlomis angustissima and Phlomis fruticosa* have shown that these plants have high phenolic content and significant antioxidant activity.[20–23] This suggests that species of the Phlomis genus can be considered natural antioxidant sources, but also demonstrates the lack of comparative data on the PP species.

Based on this deficiency, this study aims to determine the antioxidant capacity of the aerial parts of the Phlomis pungens var. pungens species, known as "silvanok" in Anatolia, using multiple analysis methods, including 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS•⁺), 2,2-diphenyl-1-picrylhydrazyl (DPPH•), and ferric reducing antioxidant power (FRAP) assays, and to assess their total phenolic and flavonoid contents. The methanol:water (7:3, v/v) solvent system used in this study was chosen because it provides effective extraction of both polar and semi-polar phenolic compounds. The results obtained are aimed at contributing to comparative phytochemical studies among Phlomis species and to expand the existing literature on the bioactive potential of this endemic species.

## METHODS

### Reagents, Instruments, and Plant Material

All chemicals used in the study were of analytical grade and were obtained from reliable commercial sources. Standard compounds Trolox (≥98%), gallic acid (≥99%), quercetin (≥95%), butylated hydroxytoluene (BHT, ≥99%), and butylated hydroxyanisole (BHA, ≥98%) were supplied by Sigma-Aldrich (St. Louis, MO, USA). Folin–Ciocalteu reagent (FCR), sodium carbonate ($Na_2CO_3$), aluminum chloride ($AlCl_3$), ammonium acetate ($NH_4CH_3COO$), potassium ferricyanide [$K_3Fe(CN)_6$], iron(III) chloride ($FeCl_3$), trichloroacetic acid (TCA), potassium persulfate ($K_2S_2O_8$), , sodium phosphate monobasic ($NaH_2PO_4$, ≥99%), and sodium phosphate dibasic ($Na_2HPO_4$, ≥99%) were supplied by Merck (Darmstadt, Germany). The phosphate buffer used in the study was prepared from these components and adjusted to pH 7.0. In addition, methanol and distilled water used in the preparation of DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) radical solutions were of analytical grade and were filtered before experimental procedures. No additional purification steps were applied to any of the reagents prior to analysis. All absorbance measurements were carried out using a Biotek Fully Automatic ELISA Reader / Microplate Spectrophotometer (Biotek Instruments, Winooski, VT, USA), equipped for absorbance detection in 6- to 384-well plates. A Vortex mixer (WISEMIX VM-10, Wisd Laboratory Instruments, Korea) was used for homogenizing solutions

during the experimental procedures. All analyses were performed in triplicate, and the results are expressed as mean values ± standard deviation (SD).

The aerial parts of PP were collected from Ergan Mountain, Erzincan, in the summer of 2024. They were characterized by taxonomist Prof. Dr. Ali Kandemir from Erzincan Binali Yıldırım University. The original specimens were deposited in Erzincan Binali Yıldırım University Herbarium (EBYU) (Herbarium No: EBYU-0000035).

**Preparation of Extracts**

The aerial parts of PP were air-dried at room temperature and homogeneously ground to a fine powder. 10 grams of plant material was macerated overnight in a methanol:water (7:3, v/v) mixture. The extraction procedure was carried out in triplicate at room temperature. After extraction, plant residue was removed, the solvents obtained were combined and the methanol was evaporated. The remaining water phase was dried by lyophilization to obtain an amorphous solid (12.9 g per plant). The final extracts were stored in dark bottles at +4 °C until use.

Methanol was chosen as the primary extraction solvent because of its strong polarity and high efficiency in dissolving a broad range of phenolic and flavonoid compounds. Among commonly used alcohol-based solvents, methanol provides superior extraction yields for phenolic acids and flavonoids, which are the main antioxidant constituents of Phlomis species. The methanol:water (7:3, v/v) solvent system was therefore selected to achieve balanced polarity and to maximize the recovery of both polar and semi-polar phytochemicals while minimizing the co-extraction of non-phenolic impurities. However, considering the toxic properties of methanol, more environmentally friendly solvent systems (e.g., eth

anol:water) are planned to be evaluated in future studies in accordance with green chemistry principles.

**Total Phenolic Content (TPC)**

TPC of the extract was determined by spectrophotometric method using Folin–Ciocalteu reagent according to Akman et al.[24]. For this purpose, plant extract and standards were prepared as stock solutions at a concentration of 1 mg/mL. 100 µL of each stock solution was taken and diluted by adding 4.5 mL of distilled water. 300 µL of 2% $Na_2CO_3$ (sodium carbonate) solution and then 100 µL of Folin–Ciocalteu reagent were added to the resulting mixture, respectively. After the mixture was homogenized in the vortex device, it was incubated at room temperature for 120 minutes in the dark. At the end of the incubation period, the absorbance values of the samples were measured with a spectrophotometer at a wavelength of 760 nm wavelength. Gallic acid was used as a reference standard. Calibration curve (y = 0.0009x + 0.0208) was obtained with different concentrations of gallic acid (1–1000 µg/mL) used for calculations. (Figure 1-A) The results were expressed in mg gallic acid equivalent phenolic content / g extract.

**Total Flavonoid Content (TFC)**

TFC of the extracts was determined by spectrophotometric method using aluminum chloride ($AlCl_3$), based on the procedures reported by Simsek.[25]. Stock solutions of extracts and standards were prepared in methanol at a concentration of 1 mg/mL. 100 µL of each stock solution was transferred to tubes and diluted by adding methanol to make the final volume 4.8 mL. Then, 100 µL of 1 M ammonium acetate ($NH_4CH_3COO$) solution and 100 µL of 10% $AlCl_3$ solution were added to the mixture, respectively, and the mixture was vortexed. The final mixture was incubated at room temperature for 45 minutes. After the incubation period, the absorbance of the mixture was measured at 415 nm wavelength. Quercetin was used as the reference compound and the results were expressed as mg quercetin equivalent / g extract (mg QE/g extract) using a calibration curve created with different quercetin concentrations in the range of 1–1000 µg/mL. (Figure 1B)

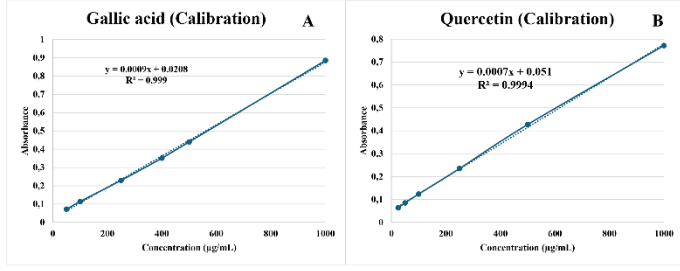



Figure 1. Calibration curves of (A) gallic acid used for TPC and (B) quercetin used for TFC.

**DPPH• Radical Scavenging Ability**

The 1,1-diphenyl-2-picrylhydrazyl (DPPH•) radical scavenging activity was evaluated following a previously established spectrophotometric method.[8] The test solution was prepared in methanol at a concentration of 0.26 mM. Using stock solutions (1 mg/mL) obtained from the tested extracts, samples were taken at different volumes (20–1000 µg/mL) and their final volumes were completed to 3 mL with methanol. 1 mL of DPPH• solution was added to these mixtures, and the final mixture was vortexed and incubated for 30 minutes at room temperature in a dark environment. At the end of the incubation period, the

absorbance of the solutions at 517 nm wavelength was measured with a spectrophotometer. The antioxidant activity against DPPH• radicals was calculated using:

Activity (%) = $[(A_{control}-A_{sample})/A_{control}]x100$,

where $A_{control}$ and $A_{sample}$ denote the absorbances of the control and test sample, respectively. Triplicate experiments were conducted, and the findings were presented as mean ± standard deviation, with $IC_{50}$ values (µg/mL) indicating antioxidant efficacy. The scavenging capacity was benchmarked against reference antioxidants, namely Trolox, BHT, BHA and ascorbic acid.

**Ferric Reducing Power (FRAP)**

The FRAP assay was conducted as previously reported by Akman et al., with slight modifications where applicable [1]. As a modification, the incubation time was extended to 20 minutes and the reaction mixture was run at 50 °C. 250 µL (0.25 mL) of the extract and isolated molecules were taken from the stock solutions at a concentration of 1 mg/mL and the volume was completed to 1.25 mL with phosphate buffer (0.2 M, pH 6.6); 1 mL of buffer was added to the solution during this process. Then, 1.25 mL of 1% potassium ferric cyanide [$K_3Fe(CN)_6$] solution was added to this mixture and vortexed, and the mixture was incubated at 50 ˚C for 20 minutes. Following the incubation step, 1.25 mL of 10% trichloroacetic acid (TCA) solution and 250 µL (0.25 mL) of 0.1% $FeCl_3$ solution were added to the reaction medium at room temperature, respectively, and the solution was vortexed to make it homogeneous. Then, the final mixture was transferred into micro plates, and the absorbance of the solutions was measured at 700 nm wavelength. In the comparison of the reducing capacity of ferric ($Fe^{3+}$) ions in FRAP analysis, Trolox, BHT, BHA and ascorbic acid were taken as reference standard antioxidants.

ABTS•+ Radical Scavenging Ability

ABTS•$^+$ activity was determined using the established protocol of Akman.[26] In this analysis, 2 mM ABTS (2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and 2.45 mM $K_2S_2O_8$ (potassium persulfate) solutions were prepared in 0.1 M phosphate buffer, pH 7.4. The prepared solutions were mixed at a 1:2 ratio and incubated for 12 hours in a dark environment. Stock solutions at a concentration of 1 mg/mL were prepared for the extracts and standards intended to be tested and taken in determined volumes (20–1000 µL) and the final volume was completed to 3 mL with phosphate buffer. 1 mL of ABTS•$^+$ solution was added to each reaction mixture, then vortexed and incubated for 60 minutes at room temperature. At the end of the incubation period, the absorbance of the reaction mixtures was measured using a spectrophotometer at a wavelength of 734 nm. Trolox, BHT, BHA and ascorbic acid were evaluated as positive controls in determining the radical scavenging capacity of ABTS•$^+$.

**RESULTS**

In this study, the antioxidant activity of methanol:water extract (7:3, v:v) of aerial parts of PP was evaluated by different methods. For this purpose, ABTS, DPPH, FRAP capacities and total phenolic and flavonoid contents of the extract were determined by spectroscopic methods. The results are presented in Table 1.

**DPPH• Free Radical Scavenging Ability**

The $IC_{50}$ value for DPPH• radical scavenging activity of the PP extract was determined as 99.02 ± 1.71 µg/mL. The $IC_{50}$ values of the standard antioxidants (trolox, BHA, BHT, and ascorbic acid) used for comparison were measured as 8.50 ± 0.77, 8.04 ± 0.69, 10.70 ± 0.73, and 9.91 ± 0.87 µg/mL, respectively. The findings indicate that the antioxidant capacity of the PP extract is significantly weaker compared to the reference compounds.

**ABTS•+ Radical Scavenging Capacity**

ABTS•$^+$ radical scavenging capacity of PP extract was determined as 36.04±3.16 µg/mL based on $IC_{50}$ value. When compared with the $IC_{50}$ values of standard antioxidants (trolox: 7.07±0.15 µg/mL, BHA: 6.33±0.19 µg/mL, BHT: 9.42±0.63 µg/mL, ascorbic acid: 8.25±0.41 µg/mL), the ability of the extract to neutralize ABTS•$^+$ radicals was determined to be approximately 4-5 times lower than that of the reference compounds.

**Table 1.** Antioxidant, TPC and TFC results of the methanol:water extract of PP.

| Samples | DPPH• $IC_{50}$ (µg/mL) | ABTS•+ $IC_{50}$ (µg/mL) | Total phenolics (mg GAE/g extract) | Total flavonoids (mg QE/g extract) | Reducing power (mg TE/g extract) |
|---|---|---|---|---|---|
| PP | 99.02±1.71 | 36.04±3.16 | 49.23±0.34 | 24.57±2.34 | 82.45±3.57 |
| Troloks | 8.50±0.77 | 7.07±0.15 | - | - | - |
| BHA | 8.04±0.69 | 6.33± 0.19 | - | - | 338.57±0.31 |
| BHT | 10.70±0.73 | 9.42±0.63 | - | - | 257.80±1.24 |
| Ascorbic acid | 9.91±0.87 | 8.25±0.41 | - | - | 394.17±0.98 |

*Values represent mean ± standard deviation (n = 3).

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### Ferric reducing antioxidant power (FRAP)

According to the results of FRAP analysis, the ferric ion reducing capacity of the extract was measured as 82.45 ± 3.57 mg TE/g extract. This value was found to be significantly low when compared with the capacities of reference antioxidants (BHA: 338.57 ± 0.31 mg TE/g extract, BHT: 257.80 ± 1.24 mg TE/g extract, ascorbic acid: 394.17 ± 0.98 mg TE/g extract). The results show that the ferric ion reducing ability of the extract is approximately 3-5 times weaker than standard antioxidants (Figure 2).

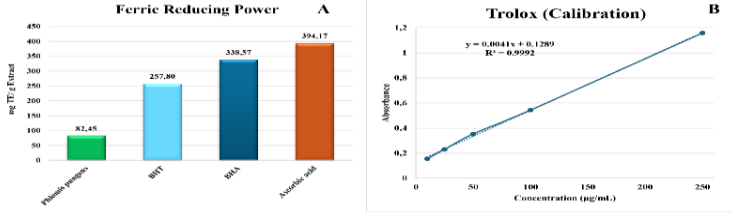



**Figure 2.** (A) The FRAP of PP extract compared with standard antioxidants (BHT, BHA, and ascorbic acid). (B) Calibration curve of Trolox used for FRAP assay.

### Total Phenolic and Flavonoid Contents

Total phenolic content was determined as 49.23 ± 0.34 mg GAE/g extract, which may indicate a moderate-to-high phenolic concentration for herbal extracts. Total flavonoid content was 24.57 ± 2.34 mg QE/g extract, indicating that the flavonoid components of the extract may provide a certain level of antioxidant activity.

This study was conducted to evaluate the antioxidant properties of a methanol:water (7:3, v/v) extract obtained from the aerial parts of PP. The antioxidant capacity of the extract was analyzed by DPPH• and ABTS•$^+$ radical scavenging tests, as well as spectrophotometric methods, considering the FRAP, total phenolic and flavonoid contents. The data obtained revealed that the extract exhibited lower antioxidant activity compared to reference antioxidant compounds.

According to DPPH• and ABTS•$^+$ test results, the radical scavenging capacity of the extract was found to be limited compared to synthetic antioxidants. However, the measured total phenolic (49.23 ± 0.34 mg GAE/g extract) and flavonoid (24.57 ± 2.34 mg QE/g extract) contents indicated that the plant has a certain antioxidant potential. Ferric reducing power was also observed to be moderate. These findings indicate that the PP is a natural source rich in phenolic compounds and can be considered a natural antioxidant source.

## DISCUSSION

The findings are generally consistent with literature studies on the antioxidant potential of the PP. A study by Taşkın et al. on the methanolic extract of *P. pungens* reported lower DPPH and ABTS radical scavenging capacities compared to synthetic antioxidants such as BHT, BHA, and ascorbic acid. Similarly, the antioxidant capacity of the natural extract in this study was observed to be lower than that of reference compounds.

Furthermore, while Taşkın et al. reported the total phenolic content of *P. pungens* as 30 mg GAE/g extract, this content was determined as 49.23 mg GAE/g extract in this study. On the other hand, a study by Keser et al. on *P. pungens* reported that the ABTS (89.8–90.6%) and DPPH (65.79–77.19%) radical scavenging capacities and ferric reducing activities of ethanol and water extracts were quite high, demonstrating significant activity compared to reference antioxidants. The findings show that the leaf and flower extracts of the plant have strong radical-scavenging effects. All three studies concluded that the antioxidant capacity of *Phlomis pungens* is directly related to the phenolic and flavonoid content of the extracts.[18,19]

The findings are generally consistent with previous studies on different Phlomis species, but also show some differences. Menevşeoğlu et al.[20] reported high total phenolic content (65.8 mg GAE/g) and strong antioxidant activity in *P. armeniaca*. Similarly, Alpay et al.[21] reported that *P. russeliana* extracts had significant antioxidant capacity and this effect was related to the high phenolic compound content. On the other hand, Tarhan et al.[22] determined high total phenolic content and strong antioxidant activity in *P. angustissima* and *P. fruticosa* species, also emphasizing that solvent polarity and ethanol-based extraction method play an important role on antioxidant activity. Compared to these studies, the moderate antioxidant capacity determined in PP extracts may be due to both phytochemical differences among species and the fact that the methanol:water (7:3, v/v) solvent system used limited the extraction efficiency of certain phenolic compounds. These results indicate that the variation in antioxidant activity observed in the Phlomis genus depends not only on genetic factors but also on environmental conditions and methodological parameters.

While the findings obtained in this study are generally consistent with those reported in the literature, some variations were observed. These differences may be due to analytical variables such as the plant part used, the extraction method (maceration), the preferred solvent type and ratio, as well as factors such as the environmental conditions in which the plant was grown and the time of collection. In particular, the quantitative and qualitative profiles of phytochemical compounds may vary depending on the extraction parameters used. However, the data obtained suggest that these variables have a decisive influence on the results.

In this study, the antioxidant capacity of a methanol:water (7:3, v/v) extract obtained from the aerial parts of *Phlomis pungens* var. *pungens* was evaluated using DPPH• and ABTS•$^{+}$ radical scavenging tests. Parameters such as total phenolics, flavonoid content, and ferric reducing power were also analyzed using spectrophotometric methods. The findings revealed that the extract exhibited lower antioxidant activity compared to reference antioxidant compounds. Furthermore, the total phenolic (49.23 mg GAE/g extract) and flavonoid (24.57 mg QE/g extract) content values obtained in this study are consistent with the moderate antioxidant capacity exhibited by the samples in DPPH and ABTS analyses. Phenolic and flavonoid compounds, especially hydroxyl groups, are known to play an active role in free radical scavenging activity. This once again demonstrates the decisive influence of phenolic concentration on antioxidant activity.

The methanol:water (7:3, v/v) solvent system used in this study was chosen because it provides high efficiency in the extraction of phenolic and flavonoid compounds and is consistent with previous phytochemical studies on Phlomis species. Because methanol has a high solubilizing capacity for phenolic compounds, this ratio provides the most effective demonstration of the plant's antioxidant potential. However, considering the toxic properties of methanol, when evaluated from the perspective of environmental chemistry and green chemistry principles, it is recommended that more environmentally friendly and sustainable solvent systems, such as ethanol:water, be considered in future studies.

The results are generally consistent with similar studies in the literature; differences may be due to variables such as the extraction method, solvent ratio, geographical region, and the period during which the plant was collected. In this regard, *Phlomis pungens* var. *pungens* can be considered a rich natural source of phenolic compounds and indicates a natural antioxidant candidate. Future bioavailability, toxicology, and in vivo/in vitro biological activity studies will be crucial to further demonstrate the pharmacological potential of this plant.

**Peer-review:** Externally peer-reviewed.
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## REFERENCES


1. Akman TC, Simsek S, Aksit Z, Aydin A, Yilmaz MA. Exploring the Potential of Psephellus huber-Marathi (Wagenitz) Wagenitz: A Comprehensive UHPLC-MS/MS Analysis of Phytochemical Composition and Evaluation of Antioxidant, Antimicrobial, and Antiproliferative Activities. *Kahramanmaraş Sütçü İmam Üniversitesi Tarım ve Doğa Derg*. 2024;27(4):782-792. [CrossRef]
2. Akşit H, Şimşek S, Aydın A, Bayar Y, Alkan M. Chemical Composition and Bioactivities of Ferulago setifolia K. Koch. Essential Oil: Antifungal, Insecticidal and Antiproliferative Potential. *Flavour Fragr J*. 2025;40(4):665-672. [CrossRef]
3. Kawamura T, Muraoka I. Exercise-Induced Oxidative Stress and the Effects of Antioxidant Intake from a Physiological Viewpoint. *Antioxidants 2018, Vol 7, Page 119*. 2018;7(9):119. [CrossRef]
4. Özli S, Senol O, Karaoğlan ES. Evaluating the Antioxidant Capacity of Rheum Ribes via Cupric Reducing Antioxidant Capacity, Ferric Reducing Antioxidant Power and 2,2-Diphenyl-1- picrylhydrazyl Methods. *Pharmata*. 2024;4(1):14-18. [CrossRef]
5. Aydın B, Alkuyruk SB, Tekman E, Yuca H, Karadayı M, Gülşahin Y, et al. Investigating the phytochemical profile, biological potentials, morphological, and anatomical characteristics of Cyclotrichium origanifolium (Labill.) Manden. & Scheng. (Lamiaceae) from Turkey. *Protoplasma*. 2025;262(2):279-298. [CrossRef]
6. Nwozo OS, Effiong EM, Aja PM, Awuchi CG. Antioxidant, phytochemical, and therapeutic properties of medicinal plants: a review. *Int J Food Prop*. 2023;26(1):359-388. doi:10.1080/10942912.2022.2157425 [CrossRef]
7. Yuca H, Aydin B, Karakaya S, Goger G, Bingöl Z, Civas A, et al. Exploring Astrodaucus orientalis (L.) Drude: Phytochemical Analysis and its Biological Potential Against Alzheimer's and Diabetes. *Chem Biodivers*. 2024;21(2):e202301753. [CrossRef]
8. Varol T, Osma E, Şimşek S, Elveren M. Antioxidant activities of plant species growing in different habitats (serpentine, gypsum and limestone). *Front Life Sci Relat Technol*. 2023;4(3):150-156. [CrossRef]
9. Gözcü S, Akşit Z, Aydın A, Yılmaz MA, Şimşek S. Comprehensive phenolic profiling and biological evaluation of Centaurea glastifolia L. (Asteraceae). *Nat Prod Res*. 2024;39(4):633-644. [CrossRef]
10. Sarikurkcu C, Uren MC, Tepe B, Cengiz M, Kocak MS. Phenolic content, enzyme inhibitory and antioxidative activity potentials of Phlomis nissolii and P. pungens var. pungens. *Ind Crops Prod*. 2014;62:333-340. [CrossRef]
11. Işık M, Dikici E, Tohma H. Biology EKHJ of, 2017 undefined. Antioxidant activity and total phenolic/flavonoid contents of phlomis pungens L. *dergipark.org.tr*. 2017;45(3):425-433. [CrossRef]
12. Saracoglu I, Suleimanov T, Shukurova A, Dogan Z. Iridoids and Phenylethanoid Glycosides from Phlomis pungens of the Flora of Azerbaijan. *Springer*. 2017;53(3):576-579. [CrossRef]
13. Sarikurkcu C, Sabih Ozer M, Cakir A, Eskici M, Mete E. GC/MS Evaluation and In Vitro Antioxidant Activity of Essential Oil and Solvent Extracts of an Endemic Plant Used as Folk Remedy in Turkey: Phlomis bourgaei Boiss. *Evidence-Based Complement Altern Med*. 2013;2013(1):293080. [CrossRef]
14. Sarikurkcu C, Ćavar Zeljković S. Chemical composition and antioxidant activity of Phlomis leucophracta, an endemic species from Turkey. *Nat Prod Res*. 2020;34(6):851-854. [CrossRef]
15. Ulukanlı Z, Akkaya A. Antibacterial activities of Marrubium catariifolium and Phlomis pungens var. hirta grown wild in Eastern Anatolia, Turkey. *Int J Agric Biol*. 2011;13(1):105-109. [CrossRef]
16. Kirimer N, Suleymanov TA, Kurkcuoglu M, Nazemiyeh H, Shukurova AS, Jouyban A. Essential Oil Constituents of Phlomis pungens Willd. from Azerbaijan. *J Essent Oil Bear Plants*. 2017;20(6):1492-1501. [CrossRef]
17. Okur ME, Karadağ AE, Reis R, Sipahi H, Arslan R, Demirci B, et al. Phytochemical and in vitro pharmacological evaluation of Phlomis pungens. *J Fac Pharm Ankara Univ*. 2022;46(1):11-22. [CrossRef]
18. Taşkın T, Çam ME, Emre G, Hazar-yavuz AN, Kabasakal L, Bitiş L. Antioxidant and anti-inflammatory activities of Phlomis pungens and Coridothymus capitatus. *J Res Pharm*. 2025;22(1):80-85. [CrossRef]
19. Keser S, Turkoglu S, Celik S, Turkoglu I. Determination of Antioxidant Capacities of Phlomis pungens Willd. var. hispida Hub.-Mor. | Asian Journal of Chemistry. *Asian J Chem*. 2012;24(6):2780. [CrossRef]
20. Menevseoglu A, Yılmaz F, Topal M, Topal F. Comprehensive Analysis of the Antioxidant and Enzyme Inhibitory Properties and Phenolic Compounds of Phlomis armeniaca: Potential Applicant in Neurodegenerative Diseases. *Chem Biodivers*. 2025;22(6):e202402969. [CrossRef]
21. [Alpay M, Dulger G, Sahin IE, Dulger B. Evaluating antimicrobial and antioxidant capacity of endemic *Phlomis russeliana* from Turkey and its antiproliferative effect on Human Caco-2 Cell Lines. *An Acad Bras Cienc*. 2019;91(3):e20180404. [CrossRef]
22. Tarhan L, Ozturk Urek R, Oner A, Nakiboglu M. Evaluation of phenolic profiles, antioxidant activities, and cytotoxic and apoptotic potentials of Phlomis angustissima and Phlomis fruticosa, medicinal plants from Turkey. *Eur J Integr Med*. 2022;55:102188. [CrossRef]
23. Sarikurkcu C, Uren MC, Tepe B, Cengiz M, Kocak MS. Phlomis armeniaca: Phenolic compounds, enzyme inhibitory and antioxidant activities. *Ind Crops Prod*. 2015;78:95-101. [CrossRef]
24. Akman TC, Simsek S, Erken İN, Yildiztekin KG, Tasci H, Türkoğlu Hİ, et al. Phytochemical Profile, Antioxidant, Anticholinesterase, and Antiproliferative Activities of Endemic Onosma discedens: In Vitro and In Silico Approaches. *Chem Biodivers*. Published online 2025:e02147. [CrossRef]
25. Şimşek S. Green Synthesis, Characterization, and Multifunctional Bioactivity of Thymus pectinatus-Derived Silver Nanoparticles. *ChemistrySelect*. 2025;10(38):e03764. [CrossRef]
26. Akman TÇ. Antioxidant activity, amino acid composition and trace element levels of Verbascum lasianthum Boiss. ex Bentham from Erzincan, Türkiye. *Türk Doğa ve Fen Derg*. 2025;14(1):146-155. [CrossRef]