FABAD J. Pharm. Sci., 50, 3, 609-620, 2025
Doi: 10.55262/fabadeczacilik.1785409
RESEARCH ARTICLE

Comparative Antioxidant Activities and Total Phenolic Content of *Hypericum perforatum*, *H. scabrum*, and *H. heterophyllum* from Different Regions of Türkiye

Osman Tuncay AGAR[*°], Bekir TOSUN[**], Tunahan DEMIRCI[***]

Comparative Antioxidant Activities and Total Phenolic Content of Hypericum perforatum, H. scabrum, and H. heterophyllum from Different Regions of Türkiye

SUMMARY

The genus Hypericum is a rich source of bioactive compounds, particularly phenolics and flavonoids, which play a key role in antioxidant activity. While H. perforatum (St. John's Wort) has been extensively investigated, comparative evaluations of Turkish representatives of the genus, including the widespread H. scabrum and the endemic H. heterophyllum, remain limited. This study compared the antioxidant capacities and total phenolic content (TPC) of these three species collected from different regions of Türkiye. Aerial parts were extracted with 70% ethanol using ultrasonic-assisted extraction, and the TPC was determined by the Folin–Ciocalteu method. Antioxidant activities were assessed by DPPH and ABTS radical scavenging assays, with Trolox as the reference standard. H. perforatum exhibited the highest TPC (201.4 ± 2.8 mg GAE/g extract) and antioxidant activity (IC_{50}: 9.82 ± 0.76 µg/mL for DPPH; 7.01 ± 0.98 µg/mL for ABTS), followed by H. heterophyllum (141.9 ± 1.9 mg GAE/g; 17.16 ± 0.67 and 10.10 ± 1.15 µg/mL). H. scabrum showed the lowest TPC (129.1 ± 2.1 mg GAE/g) and weakest activity (24.17 ± 0.65 and 9.21 ± 1.22 µg/mL). Pearson's correlation analysis revealed strong negative associations between TPC and IC_{50} values (r = –0.94 for DPPH; r = –0.90 for ABTS), confirming phenolics as the main contributors to radical scavenging potential. These findings highlight clear interspecific differences in antioxidant capacity within Hypericum, reaffirming the pharmacological value of H. perforatum and pointing to the underexplored potential of H. heterophyllum.

Keywords: *Hypericum perforatum, Hypericum scabrum, Hypericum heterophyllum, total phenolic content, DPPH, ABTS.*

Türkiye'nin Farklı Bölgelerinden Toplanan Hypericum perforatum, H. scabrum ve H. heterophyllum Türlerinin Karşılaştırmalı Antioksidan Aktiviteleri ve Toplam Fenolik İçeriği

ÖZ

Hypericum cinsi, özellikle fenolikler ve flavonoidler açısından zengin biyoaktif bileşikler içermekte olup, bu bileşikler antioksidan aktivitede önemli rol oynamaktadır. H. perforatum (Sarı Kantaron) kapsamlı şekilde araştırılmış olsa da, cinsin Türkiye'deki temsilcileri arasında yaygın olarak bulunan H. scabrum ve endemik H. heterophyllum üzerine yapılan karşılaştırmalı çalışmalar sınırlıdır. Bu çalışmada Türkiye'nin farklı bölgelerinden toplanan bu üç türün toplam fenolik içerikleri (TFİ) ve antioksidan aktiviteleri karşılaştırılmıştır. Bitkilerin toprak üstü kısımları %70 etanol ile ultrasonik destekli ekstraksiyon yöntemiyle ekstrakte edilmiş, TFİ Folin–Ciocalteu yöntemiyle belirlenmiştir. Antioksidan aktiviteler DPPH ve ABTS radikal süpürme testleri ile değerlendirilmiş, Trolox referans standart olarak kullanılmıştır. H. perforatum en yüksek TFİ'ye (201,4 ± 2,8 mg GAE/g ekstre) ve antioksidan aktiviteye (IC_{50}: DPPH için 9,82 ± 0,76 µg/mL; ABTS için 7,01 ± 0,98 µg/mL) sahip tür olmuştur. Bunu H. heterophyllum (141,9 ± 1,9 mg GAE/g; 17,16 ± 0,67 ve 10,10 ± 1,15 µg/mL) izlemiş, H. scabrum ise en düşük TFİ'ye (129,1 ± 2,1 mg GAE/g) ve en zayıf aktiviteye (24,17 ± 0,65 ve 9,21 ± 1,22 µg/mL) sahip olmuştur. Pearson korelasyon analizi, TFİ ile IC_{50} değerleri arasında güçlü negatif ilişkiler olduğunu göstermiştir (DPPH için r = –0,94; ABTS için r = –0,90). Bu sonuçlar, fenolik bileşiklerin antioksidan potansiyelin başlıca belirleyicileri olduğunu doğrulamaktadır. Bulgular, Hypericum türleri arasında belirgin antioksidan kapasite farklılıklarını ortaya koymakta, H. perforatum'un farmakolojik önemini yeniden vurgularken H. heterophyllum'un da keşfedilmemiş potansiyeline işaret etmektedir.

Anahtar Kelimeler: *Hypericum perforatum, Hypericum scabrum, Hypericum heterophyllum, toplam fenolik içerik, DPPH, ABTS.*

Recieved: 17.09.2025
Revised: 29.09.2025
Accepted: 2.10.2025

[*] ORCID: 0000-0002-1676-2443, Department of Pharmacognosy, Faculty of Pharmacy, Suleyman Demirel University, Isparta, 32260, Türkiye.
[**] ORCID: 0000-0002-2470-3865, Department of Plant and Animal Production, Burdur Vocational School of Food, Agriculture and Livestock, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye.
[***] ORCID: 0000-0001-8516-911X, Department of Pharmaceutical Biotechnology, Faculty of Pharmacy, Suleyman Demirel University, Isparta, 32260, Türkiye.

[°] Corresponding Author; Osman Tuncay AGAR
E-mail: tuncayagar@sdu.edu.tr

INTRODUCTION

The genus *Hypericum* (family Hypericaceae) comprises more than 500 species distributed worldwide, many of which have long-standing use in traditional medicine for their antidepressant, anti-inflammatory, antimicrobial, and wound-healing properties (El-Chaghaby et al., 2024; Özkan & Mat, 2013). Türkiye represents one of the main centers of *Hypericum* diversity. Floristic surveys report between 98 and 106 taxa distributed across 19–20 sections, with approximately 45–49 species being endemic to the country (Ersoy, Eroglu Ozkan, Boga, Yilmaz, & Mat, 2019; Özkan & Mat, 2013; Uskutoglu & Coşge Şenkal, 2021). This high diversity and endemism highlight the importance of Türkiye as a reservoir of *Hypericum* species and justify comparative phytochemical studies. Among these, *Hypericum perforatum* L. (St. John's Wort) is the most extensively investigated, with standardized extracts rich in phenolics, flavonoids, and phloroglucinols such as hyperforin, which are considered key contributors to its pharmacological effects, including antioxidant activity (Orcic, Mimica-Dukic, Franciskovic, Petrovic, & Jovin, 2011). In particular, the abundance of phenolic compounds in *Hypericum* is regarded as a critical determinant of bioactivity, and total phenolic content (TPC) has been widely employed as a reliable indicator of antioxidant potential in plant extracts.

Antioxidant activity in *Hypericum* species is closely linked to their secondary metabolites, particularly phenolic acids and flavonoids, which exert radical-scavenging and metal-chelating properties (Öztürk, Tunçel, & Potoğlu-Erkara, 2009). Numerous studies have shown that *H. perforatum* extracts display potent antioxidant capacity across multiple assay systems, often correlating strongly with high total phenolic content (Seyrekoglu, Temiz, Eser, & Yildirim, 2022). Other taxa distributed in Türkiye, such as *H. scabrum* and *H. heterophyllum*, have also been reported to contain diverse phenolic compounds with promising biological activity. *H. scabrum* is known to possess notable levels of phenolics and flavonoids associated with antioxidant and cytotoxic effects (Jiang et al., 2015), while *H. heterophyllum*, a species endemic to Türkiye, has demonstrated significant radical scavenging potential supported by its phenolic composition (Hazman et al., 2022; Yaman, Erenler, Atalar, Adem, & Çalişkan, 2024). These observations highlight the central role of phenolic richness, measurable through TPC assays, in determining the antioxidant potential of *Hypericum* extracts.

Although these species have each been studied individually, direct comparative evaluations across multiple *Hypericum* taxa remain limited. Most prior work has emphasized either single-species assessments or broad surveys without detailed cross-species comparisons (Özdemir, Uzun, Gül, Gül, & Çon, 2020). Furthermore, while TPC has frequently been used to estimate antioxidant potential in plants, systematic comparisons of TPC values across *Hypericum* species remain relatively rare — among the few are studies of nine *Hypericum* taxa in Greece comparing TPC/total flavonoid content (TFC) and antioxidant assays (Kakouri et al., 2023), and phenolic profiling among seven species showing consistent interspecific differences in Europe (Zdunic, Godjevac, Savikin, & Petrovic, 2017). Understanding interspecific differences is therefore crucial, as antioxidant activity may vary not only with environmental influences but also with intrinsic phytochemical diversity and total phenolic content among taxa.

In this context, the present study aimed to compare the antioxidant activities and TPC of three *Hypericum* species distributed in Türkiye—*H. perforatum*, *H. scabrum*, and *H. heterophyllum*. These taxa were evaluated using two widely applied radical scavenging assays, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), alongside TPC determination to better explain the observed bioactivities. By situating these findings within the broader literature on *Hypericum* phytochemistry, this study seeks to clarify interspecific variation in antioxidant capacity, establish correlations with phenolic content, and highlight the pharmacological relevance of lesser-studied species alongside the well-known *H. perforatum*.

MATERIAL and METHOD

Plant material

Aerial parts of three *Hypericum* species were collected from different regions of Türkiye during their flowering period. The species were identified by Dr. Osman Tuncay Agar, and voucher specimens were deposited in the Herbarium of the Department of Pharmaceutical Botany (HUEF), Hacettepe University, Ankara, Türkiye. Collection details are summarized in Table 1, and voucher specimens are preserved at HUEF for future reference. These authenticated materials provided the basis for subsequent TPC and antioxidant evaluations.

Table 1. Collection details of the *Hypericum* species investigated in this study

Species	Herbarium No.	Locality & Province	Coordinates	Altitude (m)	Habitat
HH	HUEF 14013	Ankara, Kızılcahamam District, between Gebeler and Berçinçatak Villages (5 km past Gebeler Village)	40°39′39″ N, 32°30′58″ E	1288	Rocky slopes, montane vegetation
HS	HUEF 14038	Sivas, Yeniçubuk, 2 km before Bengiser Plateau, mountain road	36°23′45″ N, 36°02′10″ E	1750	High mountain steppe
HP	HUEF 14035	Hatay, Samandağ, Çevlik, Musa Mountain, Kapısuyu Village	36°8′2.28″ N, 35°57′6.59″ E	376	Mediterranean scrub

HP: *H. perforatum*; HS: *H. scabrum*; HH: *H. heterophyllum*

Extraction

Dried aerial parts (5 g) of each *Hypericum* species were extracted with 40 mL of 70% (v/v) aqueous ethanol. This solvent system was selected for its proven efficacy in solubilizing both polar and moderately non-polar phytochemicals, particularly phenolic acids and flavonoids. Extraction was performed in an ultrasonic water bath, a method known to enhance cell wall disruption and improve the recovery of bioactive constituents, including phenolics, in *Hypericum* species (Gao, Hu, Shen, Zheng, & Liang, 2023; Tahmasebi-Boldaji, Hatamipour, Khanahmadi, Sadeh, & Najafipour, 2019).

Each sample was processed in triplicate to ensure reproducibility, and the combined extracts were concentrated under reduced pressure at 40 °C using a rotary evaporator to remove the ethanol fraction. The residues were re-dissolved in distilled water, frozen, and subsequently lyophilized to obtain dry powdered extracts suitable for both antioxidant and phenolic content analyses. The extraction yields, expressed as % (w/w) of dry extract relative to initial plant material, were 18.4% for *H. perforatum*, 15.7% for *H. heterophyllum*, and 12.9% for *H. scabrum*, with *H. perforatum* giving the highest yield. Comparable ethanol–ultrasonic–lyophilization protocols, with minor modifications in solvent ratios and extraction times, have been successfully applied in studies of *Hypericum* taxa (Charalambous et al., 2025; Kakouri et al., 2023).

Determination of total phenolic content

TPC was quantified using the Folin–Ciocalteu colorimetric method, with minor modifications from Lucas, Dalla, Boeira, Verruck, and Rosa (2022). Briefly, 0.5 mL of each 70% aqueous ethanol extract was mixed with 2.5 mL of 10% (v/v) Folin–Ciocalteu reagent and allowed to react for 5 min. Subsequently, 2.0 mL of 7.5% (w/v) sodium carbonate solution was added. The mixtures were vortexed and incubated in the dark at room temperature for 30 min.

Absorbance was measured at 765 nm using a UV–Vis spectrophotometer. A gallic acid calibration curve was employed for quantification, and results were expressed as milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g extract). All measurements were carried out in triplicate and reported as mean ± standard deviation (SD).

Although Folin–Ciocalteu is a non-specific reagent that may react with other reducing substances,

it remains one of the most widely accepted methods for estimating total phenolic levels in plant extracts. In this study, TPC determination was additionally performed to assess its relationship with antioxidant activity measured by DPPH and ABTS assays.

DPPH radical scavenging assay

The antioxidant capacity of the extracts was determined using the DPPH radical scavenging method, with minor modifications from previously described protocols for *Hypericum* species (Orcic et al., 2011; Öztürk et al., 2009). Briefly, 40 µL of each extract solution was added to 260 µL of freshly prepared DPPH methanolic solution in a 96-well microplate. The mixtures were incubated at 25 °C in the dark for 30 min, after which absorbance was measured at 517 nm using a microplate reader.

Radical scavenging activity was calculated as the percentage inhibition of DPPH relative to a blank control. IC_{50} values (µg/mL) were determined from concentration–response curves by nonlinear regression. Trolox was used as a standard antioxidant for calibration. All experiments were performed in triplicate, and results were expressed as mean ± SD.

The DPPH assay was selected due to its reproducibility and widespread application in evaluating lipophilic antioxidant activity in plant extracts. In this study, DPPH results were also compared with TPC values to assess potential correlations between phenolic levels and radical scavenging capacity.

ABTS radical cation scavenging assay

The antioxidant activity of the extracts was further assessed using the ABTS radical cation decolorization assay, with minor modifications from established protocols (Kakouri et al., 2023; Öztürk et al., 2009). Briefly, 10 µL of each extract solution was added to 290 µL of ABTS radical solution in a 96-well microplate. The mixtures were incubated at 25 °C in the dark for 6 min, after which absorbance was recorded at 734 nm using a microplate spectrophotometer.

Radical scavenging activity was expressed as percentage inhibition relative to a blank control. IC_{50} values (µg/mL) were calculated from concentration–response curves using Trolox as a standard antioxidant. All measurements were carried out in triplicate and reported as mean ± SD.

The ABTS assay was included because of its broader solubility range, allowing evaluation of both hydrophilic and lipophilic antioxidant compounds. Together with the DPPH method, it provided complementary insights into radical scavenging potential. In addition, ABTS IC_{50} values were compared with TPC to explore correlations between phenolic content and antioxidant activity.

Statistical analysis

For both DPPH and ABTS assays, Trolox was used as a reference antioxidant to generate calibration curves, and results were expressed as half-maximal inhibitory concentration (IC_{50}) values in µg/mL. IC_{50} values were obtained from concentration–response curves using a four-parameter logistic nonlinear regression model. All experiments were performed in triplicate, and data were reported as mean ± SD.

Interspecific differences in antioxidant activity were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc multiple comparison test. A significance level of $\alpha = 0.05$ was applied in all cases.

To explore the relationship between phenolic content and radical scavenging capacity, Pearson's correlation coefficients (r) were calculated between TPC values and IC_{50} values obtained from both DPPH and ABTS assays. Statistical analyses were performed using software packages suitable for nonlinear regression, correlation analysis, and post-hoc testing.

RESULTS and DISCUSSION

Total phenolic content

TPC of 70% aqueous ethanol extracts from *Hypericum perforatum*, *H. scabrum*, and *H. heterophyllum* was determined using the Folin–Ciocalteu method (Table 2). Among the three taxa, *H. perforatum* exhibited the highest TPC (201.4 ± 2.8 mg GAE/g extract), followed by *H. heterophyllum* (141.9 ± 1.9 mg GAE/g),

while *H. scabrum* showed the lowest content (129.1 ± 2.1 mg GAE/g). Statistical analysis (one-way ANOVA followed by Tukey's post-hoc test, $p < 0.05$) confirmed that all interspecific differences were significant.

These findings are consistent with previous reports, which likewise identified *H. perforatum* as phenolic-rich (Chimshirova, Karsheva, Diankov, & Hinkov, 2019) and *H. scabrum* as comparatively poorer in polyphenols (Kızıl, Kızıl, Yavuz, Emen, & Hakimoğlu, 2008). *H. heterophyllum* showed intermediate levels, similar to values reported by Yaman (2020). Such variation reflects both species-specific biosynthetic capacity and ecological influences on secondary metabolism.

Importantly, the observed ranking (*H. perforatum* > *H. heterophyllum* > *H. scabrum*) parallels trends later confirmed in antioxidant assays, supporting TPC as a reliable marker of radical scavenging potential in *Hypericum* extracts (Öztürk et al., 2009; Zdunic et al., 2017).

Table 2. Total phenolic content (TPC) of *Hypericum* species.

Species	TPC (mg GAE/g extract)
H. perforatum	201.4 ± 2.8[a]
H. heterophyllum	141.9 ± 1.9[b]
H. scabrum	129.1 ± 2.1[c]

Values are expressed as mean ± SD (n ≥ 3). Different superscript letters (a–c) indicate statistically significant differences between species according to Tukey's post-hoc test ($p < 0.05$).

Antioxidant activity

The antioxidant activities of *H. perforatum* (HP), *H. scabrum* (HS), and *H. heterophyllum* (HH) were evaluated using DPPH and ABTS radical scavenging assays, with IC_{50} values summarized in Table 3 and Figure 1. Trolox was used as the standard reference.

In the DPPH assay, HP exhibited the strongest activity (9.82 ± 0.76 µg/mL), followed by HH (17.16 ± 0.67 µg/mL), while HS was the least active (24.17 ± 0.65 µg/mL). Trolox, as expected, displayed the highest potency (3.72 ± 0.43 µg/mL).

In the ABTS assay, a similar trend was observed, though the interspecific differences were narrower: HP (7.01 ± 0.98 µg/mL) remained the most active, while HS (9.21 ± 1.22 µg/mL) and HH (10.10 ± 1.15 µg/mL) did not differ significantly from each other. Overall, IC_{50} values were consistently lower in ABTS compared to DPPH, reflecting higher assay sensitivity.

Table 3. IC_{50} values (µg/mL, mean ± SD, n ≥ 3) of *Hypericum* species and Trolox in DPPH and ABTS assays

Assay	*HP*	*HS*	*HH*	Trolox
DPPH	9.82 ± 0.76[a]	24.17 ± 0.65[c]	17.16 ± 0.67[b]	3.72 ± 0.43[d]
ABTS	7.01 ± 0.98[a]	9.21 ± 1.22[b]	10.10 ± 1.15[b]	2.25 ± 0.66[c]

HP: *H. perforatum*; HS: *H. scabrum*; HH: *H. heterophyllum.* Different superscript letters (a–d) indicate statistically significant differences between species according to Tukey's post-hoc test ($p < 0.05$).

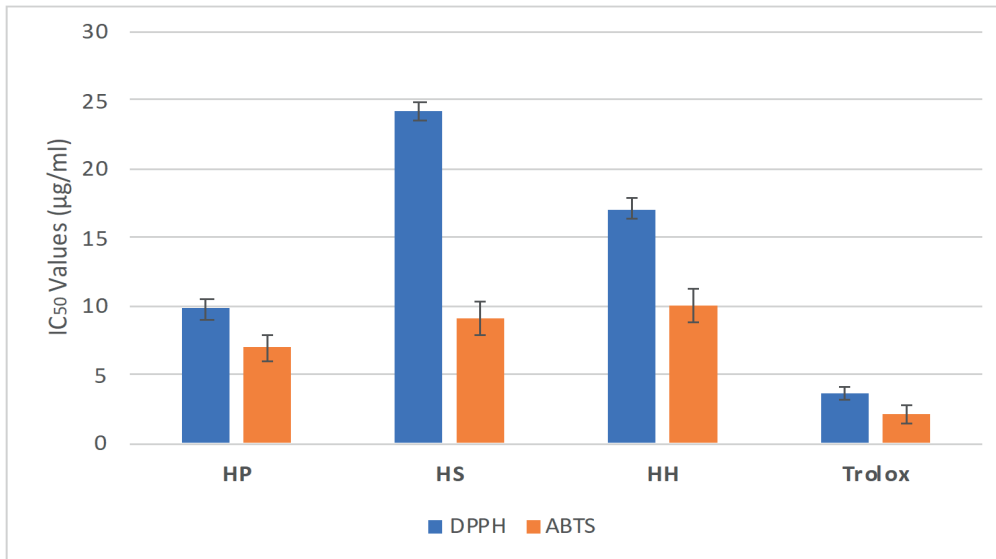

Figure 1. IC_{50} values (µg/mL) of *Hypericum perforatum* (HP), *H. scabrum* (HS), *H. heterophyllum* (HH), and Trolox determined by DPPH and ABTS assays. Data are expressed as mean ± SD (n ≥ 3).

The observed ranking of antioxidant potency (HP > HH > HS) corresponds well with the known phytochemical richness of these taxa. *H. perforatum* is widely recognized as a phenolic- and flavonoid-rich species, containing chlorogenic acid, caffeic acid, hyperoside, rutin, and quercetin derivatives, all of which are strongly associated with radical scavenging capacity (Orcic et al., 2011; Öztürk et al., 2009). Its dominance in antioxidant assays has also been demonstrated in other comparative studies of Turkish *Hypericum*, where *H. perforatum* consistently outperformed co-occurring taxa (Seyrekoglu et al., 2022; Zdunic et al., 2017).

By contrast, *H. scabrum* is generally reported to contain lower concentrations of total phenolics and flavonoids, consistent with its weaker activity observed here. Previous phytochemical analyses identified modest levels of hyperoside and quercetin glycosides in *H. scabrum* (Jiang et al., 2015), and studies of other Turkish species show similar lower-level phenolic profiles (Cirak et al., 2017; Özdemir et al., 2020). Although *H. scabrum* has demonstrated other pharmacological properties, such as cytotoxic and antimicrobial activities (Stojanovic, Dordevic, & Smelcerovic, 2013), its antioxidant potential appears comparatively limited.

H. heterophyllum occupied an intermediate position, displaying higher activity than HS but lower than HP. Recent work on *H. heterophyllum* flowers has shown significant TPC and TFC, with strong antioxidant activity in DPPH, ABTS, superoxide, and hydroxyl radical assays (Erenler, Yaman, Demirtas, & Hakki Alma, 2023; Eruygur et al., 2024). This species' phytochemical profile, including the presence of chlorogenic acid and quercetin derivatives (Hazman et al., 2022; Yaman et al., 2024), likely underlies its moderate antioxidant profile. This balanced composition suggests that *H. heterophyllum* may represent a promising but underexplored source of natural antioxidants.

A clear methodological distinction was also evident between the two radical scavenging assays. IC_{50} values were consistently lower in the ABTS assay than in the DPPH assay, reflecting greater sensitivity of ABTS under the tested conditions. This is consistent with the physicochemical properties of the radicals employed. DPPH is a stable nitrogen-centered radical soluble primarily in organic solvents, and thus more suitable for assessing lipophilic antioxidants (Orcic

et al., 2011; Öztürk et al., 2009). By contrast, ABTS can be dissolved in both aqueous and organic media, allowing it to interact with a broader spectrum of hydrophilic as well as lipophilic compounds (Zheleva-Dimitrova, Nedialkov, & Kitanov, 2010).

Similar patterns have been reported in other studies of *Hypericum*. In Eruygur et al. (2024), extracts of *H. heterophyllum* showed significantly lower (i.e., more potent) IC_{50} values in ABTS compared to DPPH, alongside high phenolic/flavonoid contents. In a more recent study of *H. scabrum* leaves and flowers, multiple assay methods, including DPPH and ABTS, similarly suggested that ABTS may pick up polar antioxidants more efficiently (Muhammad, 2019). Studies on *H. empetrifolium* and *H. lydium* also reveal assay-dependent variations, with ABTS often giving more favorable (lower) values than DPPH (Sut et al., 2025).

Taken together, these observations emphasize the importance of employing multiple radical scavenging assays when evaluating antioxidant potential. While both DPPH and ABTS confirmed the same activity ranking (HP > HH > HS), their complementary sensitivities provided a more complete view of the antioxidant spectrum in *Hypericum* extracts (Zdunic et al., 2017).

Correlation between TPC and antioxidant activity

A strong inverse relationship was observed between TPC and antioxidant capacity, as measured by IC_{50} values (Figure 2). Pearson's correlation coefficients were r = –0.94 for TPC versus DPPH IC_{50} and r = –0.90 for TPC versus ABTS IC_{50}, both indicating strong negative associations. These findings demonstrate that higher phenolic concentrations were consistently associated with stronger radical-scavenging activity across the tested *Hypericum* species.

Comparable results have been obtained in several recent studies. For example, *Hypericum perforatum* under chitosan treatment showed increases in both TPC and DPPH radical scavenging activity in mild water stress conditions (Amooaghaie & Rajaie, 2025). Błońska-Sikora, Zielińska, Dobros, Paradowska, and Michalak (2025) compared different commercial *H. perforatum* extracts and found a strong correlation between TPC/TFC and antioxidant assays like DPPH and FRAP. In *H. spectabile* optimized extracts, Gürgen, Sevindik, Krupodorova, Uysal, and Unal (2024) reported that phenolic compound abundance (including caffeic acid and quercetin derivatives) was mirrored by high antioxidant activity. Studies in other *Hypericum* taxa, such as *H. cordifolium* and *H. repens*, similarly underscore the relationship between phenolic/flavonoid levels and radical scavenging ability (Sapkota, Maharjan, Tiwari, & Rajbhandari, 2024).

This supports the role of phenolic compounds as key chemotaxonomic and pharmacological markers within the genus. Notably, the slightly stronger correlation with the DPPH assay in our data suggests that phenolics in these taxa may include both hydrophilic and moderately lipophilic molecules, which contribute differentially depending on radical type and assay environment.

Collectively, these results highlight TPC not only as a reliable predictor of antioxidant potential but also as a valuable screening parameter for guiding further phytochemical and pharmacological studies of *Hypericum* species.

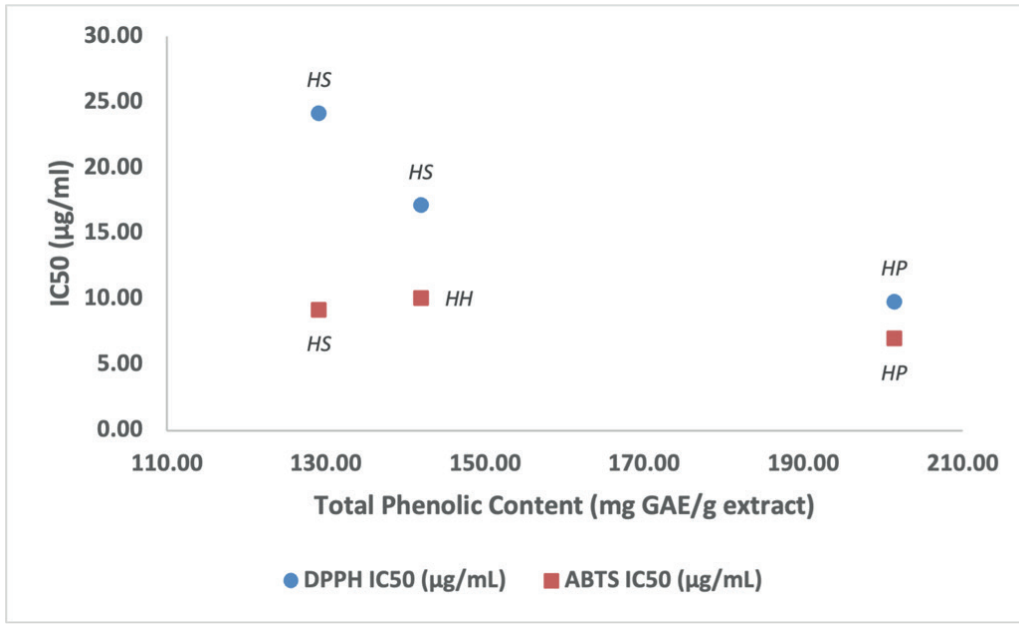

Figure 2. Correlation between total phenolic content (TPC, mg GAE/g extract) and antioxidant activity (IC_{50} values for DPPH and ABTS assays) in *H. perforatum* (HP), *H. scabrum* (HS), and *H. heterophyllum* (HH). Pearson's correlation coefficients were r = –0.94 (DPPH) and r = –0.90 (ABTS), both indicating strong negative associations. Data represent mean ± SD (n ≥ 3).

Limitations and future perspectives

While this study provides valuable comparative insights into the antioxidant potential of *H. perforatum*, *H. scabrum*, and *H. heterophyllum*, several limitations should be acknowledged. First, plant material was collected from single sites for each taxon. This constitutes a major limitation, as secondary metabolite accumulation in *Hypericum* species is strongly influenced by environmental and genetic factors such as altitude, soil structure, and climatic conditions. Consequently, the present findings should be interpreted with caution, as they may not fully represent the phytochemical and antioxidant profiles of these species across their broader geographical distribution. Previous studies have demonstrated substantial intraspecific variation in *Hypericum*, with phenolic content and antioxidant activity differing among populations depending on altitude, soil type, and climatic factors (Cirak et al., 2017; Özdemir et al., 2020). Similarly, Şanli et al. (2015) reported marked location-dependent differences in hypericin content and quality traits of Turkish *Hypericum*, further emphasizing the need for broader sampling. Broader multi-site and multi-seasonal sampling would therefore enhance the robustness of future comparative analyses.

Second, antioxidant capacity was assessed using only two radical scavenging assays (DPPH and ABTS). Although complementary, these methods capture only part of the antioxidant spectrum. Additional electron-transfer and hydrogen-donating assays, such as FRAP, CUPRAC, and ORAC, would provide a more comprehensive picture of redox-related activity (Zdunic et al., 2017).

Third, this work focused on bioactivity assays with only TPC analysis. Although TPC is a valuable screening parameter, it does not identify which specific metabolites are primarily responsible for the observed antioxidant activity. The absence of TFC measurements and compound-level profiling (e.g., HPLC-DAD or LC-MS/MS quantification of hyperoside, rutin, chlorogenic acid, quercetin derivatives) is a significant limitation. Therefore, while our results

provide useful comparative insights, they should be interpreted cautiously. Future studies should integrate TFC assays and chromatographic analyses to establish stronger correlations between specific metabolites and radical scavenging activity.

Future research should therefore integrate expanded chemical profiling, broader assay coverage, and multi-site ecological sampling to better capture the complexity of antioxidant diversity in *Hypericum*. Such integrative approaches will support the rational development of these species as pharmacologically relevant sources of natural antioxidants.

In summary, this study provides new comparative insights into interspecific differences in phenolic content and radical scavenging capacity among *H. perforatum*, *H. scabrum*, and the endemic *H. heterophyllum*. By including this underexplored taxon, our findings extend the phytochemical and pharmacological knowledge of Turkish *Hypericum* and strengthen the chemotaxonomic understanding of the genus.

CONCLUSION

This study comparatively evaluated the antioxidant activities of *Hypericum perforatum*, *H. scabrum*, and *H. heterophyllum* collected from different regions of Türkiye. Both DPPH and ABTS radical scavenging assays demonstrated clear interspecific differences, with *H. perforatum* exhibiting the strongest activity, *H. heterophyllum* showing intermediate activity, and *H. scabrum* being the least active. These results were consistent with TPC, which correlated strongly and negatively with IC_{50} values ($r = -0.94$ for DPPH; $r = -0.90$ for ABTS), confirming phenolics as key contributors to the observed antioxidant potential.

While the study was limited by single-site sampling and the use of only two radical scavenging assays, the findings provide a valuable foundation for future investigations. Expanding chemical profiling to include flavonoid quantification and metabolite fingerprinting, alongside additional antioxidant assays, will be essential for clarifying structure–activity relationships and ecological influences within the genus.

In summary, this study contributes novel comparative data by linking phenolic content with antioxidant activity across three Turkish *Hypericum* taxa. The results reaffirm the pharmacological relevance of *H. perforatum* while also highlighting the underexplored potential of the endemic *H. heterophyllum* as a promising source of natural antioxidants.

AUTHOR CONTRIBUTION STATEMENT

Conceptualization: O.T.A.; Investigation: O.T.A., B.T., T.D.; Methodology: O.T.A., B.T., T.D.; Validation: O.T.A.; Formal analysis: O.T.A., B.T.; Data curation: O.T.A., T.D.; Visualization: O.T.A.; Writing – original draft: O.T.A.; Writing – review & editing: O.T.A., B.T., T.D.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

Amooaghaie, R., & Rajaie, N. (2025). Exploring effect of chitosan on antioxidant system and hypericin content in *Hypericum perforatum* L. under various irrigation regimes. *Bmc Plant Biology, 25*(1), 799. doi:10.1186/s12870-025-06887-y

Błońska-Sikora, E., Zielińska, A., Dobros, N., Paradowska, K., & Michalak, M. (2025). Polyphenol and Flavonoid Content and Antioxidant Activity of *Hypericum perforatum* L. (St. John's Wort) Extracts for Potential Pharmaceutical and Cosmetic Applications. *Applied Sciences, 15*(5), 2590. doi:10.3390/app15052590

Charalambous, D., Kakouri, E., Ververis, A., Zorba, I., Chatzidakis, D., Andreou, M., . . . Tarantilis, P. A. (2025). Cyprus St. John's Wort, *Hypericum* repens L.: Major Constituents, Antioxidant, Antimicrobial, and Anticholinesterase Activities. *Plants, 14*(12), 1881. doi:10.3390/plants14121881

Chimshirova, R., Karsheva, M., Diankov, S., & Hinkov, I. (2019). Extraction of valuable compounds from Bulgarian St. John's Wort (*Hypericum perforatum* L.). Antioxidant capacity and total polyphenolic content. *Journal of Chemical Technology and Metallurgy, 54*(5), 952-961. Retrieved from http://dl.uctm.edu/journal/

Cirak, C., Radusiene, J., Jakstas, V., Ivanauskas, L., Seyis, F., & Yayla, F. (2017). Altitudinal changes in secondary metabolite contents of *Hypericum androsaemum* and *Hypericum polyphyllum*. *Biochemical Systematics and Ecology, 70*, 108-115. doi:10.1016/j.bse.2016.11.006

El-Chaghaby, G. A., Mohammed, F. S., Rashad, S., Uysal, I., Koçer, O., Lekesiz, Ö., . . . Sevindik, M. (2024). Genus *Hypericum*: General Properties, Chemical Contents and Biological Activities. *Egyptian Journal of Botany, 64*(1), 1-26. doi:10.21608/ejbo.2023.217116.2378

Erenler, R., Yaman, C., Demirtas, l., & Hakki Alma, M. (2023). Phytochemical investigation of *Hypericum heterophyllum* flowers: LC-ESI-MS/MS analysis, total phenolic and flavonoid contents, antioxidant activity. *The Natural Products Journal, 13*(7), 37-45. doi:10.2174/2210315513666230112165545

Ersoy, E., Eroglu Ozkan, E., Boga, M., Yilmaz, M. A., & Mat, A. (2019). Anti-aging potential and anti-tyrosinase activity of three *Hypericum* species with focus on phytochemical composition by LC–MS/MS. *Industrial Crops and Products, 141*, 111735. doi:10.1016/j.indcrop.2019.111735

Eruygur, N., Uçar, E., Tüzün, B., Ataş, M., İnanır, M., Demirbaş, A., . . . Uskutoğlu, T. (2024). Evaluation of antioxidant, antimicrobial, enzyme inhibition activity, and cell viability capacity of *Hypericum heterophyllum* Vent., an endemic species in Turkey's Flora. *Journal of Molecular Structure, 1307*, 137908. doi:10.1016/j.molstruc.2024.137908

Gao, J., Hu, D., Shen, Y., Zheng, Y., & Liang, Y. (2023). Optimization of ultrasonic-assisted polysaccharide extraction from Hyperici Perforati Herba using response surface methodology and assessment of its antioxidant activity. *International Journal of Biological Macromolecules, 225*, 255-265. doi:10.1016/j.ijbiomac.2022.10.260

Gürgen, A., Sevindik, M., Krupodorova, T., Uysal, I., & Unal, O. (2024). Biological activities of *Hypericum spectabile* extract optimized using artificial neural network combined with genetic algorithm application. *BMC Biotechnology, 24*(1), 83. doi:10.1186/s12896-024-00914-w

Hazman, Ö., Aksoy, L., Büyükben, A., Kara, R., Kargıoğlu, M., Cigerci, İ. H., & Yılmaz, M. A. (2022). LC-MS/MS profiles, multi-element levels and biological activities of *Hypericum heterophyllum* Vent. *Indian Journal of Experimental Biology (IJEB), 60*(10), 743-752. doi:10.56042/ijeb.v60i10.55070

Jiang, L., Numonov, S., Bobakulov, K., Qureshi, M. N., Zhao, H., & Aisa, H. A. (2015). Phytochemical Profiling and Evaluation of Pharmacological Activities of *Hypericum scabrum* L. *Molecules, 20*(6), 11257-11271. doi:10.3390/molecules200611257

Kakouri, E., Daferera, D., Trigas, P., Charalambous, D., Pantelidou, M., Tarantilis, P. A., & Kanakis, C. D. (2023). Comparative Study of the Antibacterial Activity, Total Phenolic and Total Flavonoid Content of Nine *Hypericum* Species Grown in Greece. *Applied Sciences, 13*(5), 3305. doi:10.3390/app13053305

Kızıl, G., Kızıl, M., Yavuz, M., Emen, S., & Hakimoğlu, F. (2008). Antioxidant Activities of Ethanol Extracts of *Hypericum triquetrifolium* and *Hypericum scabroides*. *Pharmaceutical Biology, 46*(4), 231-242. doi:10.1080/13880200701739363

Lucas, B. N., Dalla, N., Boeira, C. P., Verruck, S., & Rosa, C. S. d. (2022). Determination of total phenolic compounds in plant extracts via Folin-Ciocalteu's method adapted to the usage of digital images. *Food Science and Technology, 42*, e35122. doi:10.1590/fst.35122

Muhammad, K. (2019). Assessment of phytochemical content and antioxidant activity of the extracts collected from the leaves and flowers of *Hypericum scabrum*. *Tikrit Journal for Agricultural Sciences, 19*(1), 93-102. doi:10.25130/tjas.19.1.10

Orcic, D. Z., Mimica-Dukic, N. M., Franciskovic, M. M., Petrovic, S. S., & Jovin, E. D. (2011). Antioxidant activity relationship of phenolic compounds in *Hypericum perforatum* L. *Chem Cent J, 5*, 34. doi:10.1186/1752-153X-5-34

Özdemir, N., Uzun, F., Gül, L. B., Gül, O., & Çon, A. H. (2020). Assessment of phenolic content, antioxidant properties and antimicrobial activity of flower and leaf extracts from some *Hypericum* species affected by wild habitat altitude. *Alinteri Journal of Agriculture Science, 35*(1), 62-68. doi:10.28955/alinterizbd.739372

Özkan, E. E., & Mat, A. (2013). An overview on *Hypericum* species of Turkey. *Journal of Pharmacognosy and Phytotherapy, 5*(3), 38-46. doi:10.5897/JPP2013.0260

Öztürk, N., Tunçel, M., & Potoğlu-Erkara, İ. (2009). Phenolic compounds and antioxidant activities of some *Hypericum* species: A comparative study with *H. perforatum*. *Pharmaceutical Biology, 47*(2), 120-127. doi:10.1080/13880200802437073

Şanli, A., Karadoğan, T., Tosun, B., Özçelik, H., Muca, B., Koca, A., & Güldali, F. (2015). Göller Yöresinde Yayılış Gösteren *Hypericum* L. Taksonlarının Bazı Tarımsal ve Kalite Özellikleri. *Biyoloji Bilimleri Araştırma Dergisi, 8*(2), 54-57. Retrieved from https://bibad.gen.tr/index.php/bibad

Sapkota, S., Maharjan, A., Tiwari, S., & Rajbhandari, M. (2024). Phytochemical Analysis, Antioxidant Potential and Antibacterial Activities of Different Anatomical Parts of *Hypericum cordifolium* Choisy. *ScientificWorldJournal, 2024*, 8128813. doi:10.1155/2024/8128813

Seyrekoglu, F., Temiz, H., Eser, F., & Yildirim, C. (2022). Comparison of the antioxidant activities and major constituents of three *Hypericum* species (*H. perforatum, H. scabrum* and *H. origanifolium*) from Turkey. *South African Journal of Botany, 146*, 723-727. doi:10.1016/j.sajb.2021.12.012

Stojanovic, G., Dordevic, A., & Smelcerovic, A. (2013). Do other *Hypericum* species have medical potential as St. John's wort (*Hypericum perforatum*)? *Current Medicinal Chemistry, 20*(18), 2273-2295. doi:10.2174/0929867311320180001

Sut, S., Dall'Acqua, S., Flores, G. A., Cusumano, G., Koyuncu, İ., Yuksekdag, O., . . . Zengin, G. (2025). *Hypericum empetrifolium* and *H. lydium* as Health Promoting Nutraceuticals: Assessing Their Role Combining In Vitro In Silico and Chemical Approaches. *Food Science & Nutrition, 13*(4), e70053. doi:10.1002/fsn3.70053

Tahmasebi-Boldaji, R., Hatamipour, M.-S., Khanahmadi, M., Sadeh, P., & Najafipour, I. (2019). Ultrasound-assisted packed-bed extraction of hypericin from *Hypericum perforatum* L. and optimization by response surface methodology. *Ultrasonics Sonochemistry, 57*, 89-97. doi:10.1016/j.ultsonch.2019.05.018

Uskutoglu, T., & Coşge Şenkal, B. (2021). *Hypericum* taxa of Turkey's flora and intra-population variation of morpho-agronomic traits in *H. heterophyllum* Vent., an endemic species. *Journal of the Institute of Science and Technology, 11*(1), 743-752. doi:10.21597/jist.795693

Yaman, C. (2020). Phytochemicals and antioxidant capacity of wild growing and in vitro Hypericum heterophyllum. *Romanian Biotechnological Letters*, 25(6), 2111–2117. doi:10.25083/rbl/25.6/2111.2117

Yaman, C., Erenler, R., Atalar, M. N., Adem, Ş., & Çalişkan, U. K. (2024). Phytochemical properties, antioxidant and in vitro/in silico anti-acetylcholinesterase activities of hypericum heterophyllum leaf from Türkiye. *Brazilian Archives of Biology and Technology, 67*, e24230043. Retrieved from https://www.scielo.br/j/babt/

Zdunic, G., Godjevac, D., Savikin, K., & Petrovic, S. (2017). Comparative analysis of phenolic compounds in seven *Hypericum* species and their antioxidant properties. *Natural Product Communications, 12*(11), 1805-1811. doi:10.1177/1934578X1701201140

Zheleva-Dimitrova, D., Nedialkov, P., & Kitanov, G. (2010). Radical scavenging and antioxidant activities of methanolic extracts from *Hypericum* species growing in Bulgaria. *Pharmacognosy Magazine, 6*(22), 74-78. doi:10.4103/0973-1296.62889