e-ISSN: 2687-6698 **Research Article**

Turkish Journal of

Analytical Chemistry

TurkJAC

https://dergipark.org.tr/tr/pub/turkjac

Phenolic composition and antioxidant properties in different organs of *Hypericum elongatum* and *Hypericum orientale*

Cansu Altuntaş

Medicinal- Aromatic Plants Application and Research Centre, Artvin Coruh University, 08010, Artvin, Türkiye

Abstract

This study provides a comprehensive assessment of the phenolic composition and antioxidant capacity of two little-known *Hypericum* species, *H. elongatum* and *H. orientale*. Methanolic extracts from flowers, leaves, and stems were analyzed for total phenolic content, total flavonoid content, and antioxidant potential using 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC) assays. Fifteen phenolic compounds, including hypericin, were identified and quantified by high-performance liquid chromatography with diode array detection (HPLC-DAD).

Significant organ-specific differences were observed. The highest total phenolic content (37.87 mg GAE/g) and total flavonoid content (10.66 mg QE/g) were detected in *H. orientale* flowers. The strongest overall antioxidant activity was found in *H. elongatum* leaves (DPPH IC_{50} = 0.02 mg/mL, FRAP = 299 μmol, CUPRAC = 0.57 mmol). In *H. orientale*, DPPH scavenging was uniform across organs (IC_{50} = 0.04-0.06 mg/mL), whereas FRAP/CUPRAC values showed organ-specific differences. HPLC analyses revealed that catechin (1769 mg/kg) and epicatechin (2587 mg/kg) were dominant in *H. elongatum* leaves, comprising 42% of total phenolics. *H. elongatum* flowers contained the highest vanillic acid (3169 mg/kg) and rutin (223 mg/kg). The highest quercetin (1314 mg/kg) and hypericin (27.5 mg/kg) were detected in *H. orientale* flowers. Most remarkably, *H. orientale* stems exhibited exceptional reducing power (FRAP = 192 μmol, CUPRAC = 0.40 mmol) despite the lowest total phenolic content (22.74 mg GAE/g). This paradox is explained by pyrogallol (705 mg/kg), whose three ortho-hydroxyl groups confer superior electron-donating capacity.

These findings demonstrate that antioxidant capacity depends on specific compound identity and emphasize the importance of organ-specific harvesting strategies. *H. elongatum* leaves and *H. orientale* flowers represent complementary sources for targeted phytotherapeutic applications.

Keywords: *Hypericum*, hypericin, antioxidant, HPLC, phenolic

1. Introduction

The genus *Hypericum* L. (Hypericaceae) comprises approximately 500 herbaceous and shrubby species, many of which have long been valued in traditional medicine for their therapeutic properties. Türkiye is recognized as a major center of biodiversity for this genus, harboring nearly 100 taxa, almost half of which are endemic [1–3]. Among these, *Hypericum perforatum* L. (St. John's wort) is by far the most extensively studied species, owing to its broad spectrum of pharmacological activities, including antidepressant, anti-inflammatory, antimicrobial, and antiproliferative effects. These biological properties are primarily attributed to its complex secondary metabolite profile, which encompasses naphthodianthrones, phloroglucinols, flavonoids, and phenolic acids [4,5].

Despite the growing interest in *Hypericum*, other species such as *Hypericum elongatum* Rchb. and *Hypericum orientale* L. remain comparatively underexplored. Both taxa are native to Türkiye and parts of Eurasia, where they are occasionally employed in folk medicine; however, detailed information regarding their phytochemical composition and biological potential remains limited. Recent investigations into Turkish *Hypericum* species have revealed substantial interspecific and organ-specific variation in secondary metabolites [6], thereby emphasizing the need for systematic investigation of less-studied taxa such as *H. elongatum* and *H. orientale*.

H. elongatum is known to produce essential oils rich in monoterpenes, particularly α-pinene and β-pinene, which may contribute to its antimicrobial activity [7,8].

Citation: C. Altuntaş, Phenolic composition and antioxidant properties in different organs of *Hypericum elongatum* and *Hypericum orientale*, Turk J Anal Chem, 8(1), 2026, 9–19.

***Author of correspondence:** cansualtuntas@artvin.edu.tr
Tel: +90 (466) 215 10 00 **Fax:** +90 (466) 215 10 55
Received: November 07, 2025 **Accepted:** January 01, 2026
https://doi.org/10.51435/turkjac.1819405

Its aerial parts also contain flavonoids including catechin, epicatechin, and quercetin glycosides, suggesting notable antioxidant potential [9]. However, the presence and tissue distribution of hallmark *Hypericum* constituents, particularly hypericin and hyperforin, remain insufficiently documented. Hypericin, a naphthodianthrone derivative, serves as a critical biomarker for *Hypericum* species due to its photodynamic, antidepressant, and antitumor activities [10,11]. Analysis of hypericin is essential not only for pharmacological evaluation but also for chemotaxonomic classification, as its content varies significantly among species (ranging from 0.009% to 0.512%) and correlates with infrageneric phylogenetic advancement [12]. Despite its pharmacological and taxonomic significance, comprehensive organ-specific hypericin profiling has not yet been conducted for *H. elongatum*.

Similarly, *H. orientale* exhibits promising chemical diversity. Extracts of this species have been reported to contain hypericin, pseudohypericin, chlorogenic acid, rutin, and kaempferol [13,14]. Its essential oil composition, characterized by a high proportion of sesquiterpenes (e.g., β-caryophyllene and β-selinene), distinguishes it from other Turkish species [8]. Although hypericin has been detected in *H. orientale*, quantitative determination and organ-specific distribution patterns remain largely unexplored [12]. These preliminary findings suggest that *H. orientale* may possess unique phytochemical characteristics; however, systematic organ-level comparisons are currently lacking.

Both *H. elongatum* and *H. orientale* have ethnobotanical relevance. *H. orientale*, locally known as "çay otu," is traditionally used in eastern Anatolia for the treatment of gastrointestinal disorders and hemorrhoids [15]. Ethnomedicinal documentation for *H. elongatum* is more limited; however, its geographical overlap with other medicinal *Hypericum* species suggests that it may also be empirically utilized in traditional practices [16]. Nevertheless, such applications remain largely unverified, reinforcing the necessity for scientific evaluation of their phytochemical profiles and biological properties.

Although several studies have examined individual compounds or crude extracts from various *Hypericum* species, no research to date has conducted a simultaneous organ-specific assessment of the phenolic composition and antioxidant capacity of *H. elongatum* and *H. orientale*. Furthermore, existing reports frequently lack standardized extraction procedures and quantitative chromatographic comparisons, making it difficult to draw meaningful interspecific conclusions. This knowledge gap limits our understanding of the biological significance, pharmacological potential, and chemotaxonomic value of these taxa.

The present study addresses these gaps by providing the first comprehensive comparative evaluation of the phenolic composition and antioxidant activity across flower, leaf, and stem extracts of *H. elongatum* and *H. orientale*. Total phenolic and flavonoid contents (TPC, TFC) were determined spectrophotometrically; antioxidant capacity was assessed using three complementary assays (2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), and cupric reducing antioxidant capacity (CUPRAC)); and thirteen phenolic compounds were identified and quantified via high-performance liquid chromatography with diode array detection (HPLC-DAD). By integrating organ-specific antioxidant profiling with quantitative chemical characterization, this study provides novel insights into the phytochemical diversity of these two underexplored species and establishes a scientific foundation for their potential utilization as natural antioxidant sources in phytotherapeutic applications.

2. Materials and methods

2.1. Plant material and identification

The plant materials of the *H. elongatum and H. orientale* were collected from natural habitats in Artvin Province, Türkiye (Fig. 1a, Fig 1b). Taxonomic identification of the

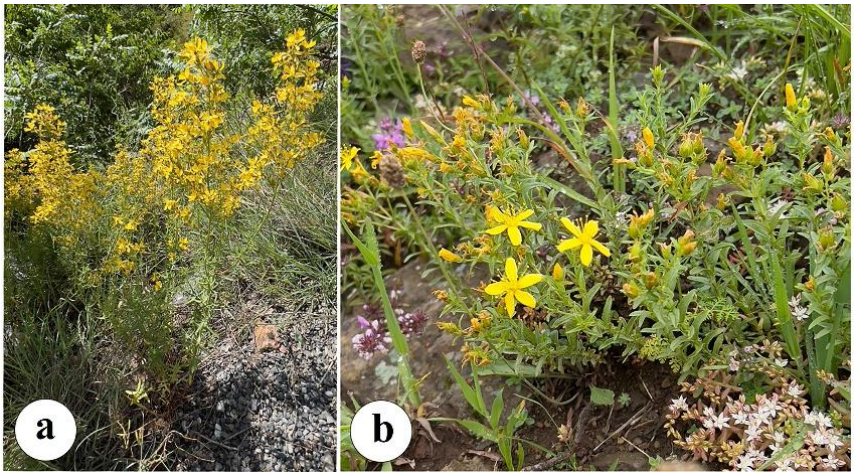

Figure 1. Habit of *Hypericum.* a- *H. elongatum* Rchb, b- *H. orientale* L.

specimens was verified using standard *Hypericum* keys proposed by Robson [17] and Ekim [18]. Voucher specimens were deposited at Artvin Çoruh University Medicinal and Aromatic Plants Application and Research Centre. Details of the taxa and collection data are provided in Table 1.

Table 1. Localities and voucher numbers of *Hypericum* species studied

Taxon	Locality	Voucher
H. elongatum Rchb.	Artvin: Yusufeli, Kılıçkaya, 1570 m, 11 June 2024	Aksu 569
H. orientale L.	Artvin: Ardanuç, Bilbilan Pleteau, 2250 m, 20 July 2022	Aksu 387

2.2. Preparation of extracts

Methanol was selected as the extraction solvent due to its exceptional capability to extract both polar and non-polar phytoconstituents, ensuring comprehensive recovery of bioactive compounds. The extraction methodology was developed through optimization of protocols adapted from [19], [20]. Dried plant material (10 g) was extracted with 100 mL of methanol (HPLC grade, Merck, Germany) maintaining a material-to-solvent ratio of 1:10 (w/v). The extraction process was initiated using ultrasonic-assisted extraction (Fisherbround, UK) operating at 600W for 30 min to facilita te initial cell wall disruption and compound solubilization. Subsequently, the mixtures were incubated in an orbital shaker (Heidelph Unimax 1010 Inkubator 1000, Germany) at 150 rpm for 24 h at room temperature under light-excluding conditions to maximize extraction efficiency and prevent photodegradation of sensitive compounds. Upon completion of the incubation period, a comprehensive two-stage filtration procedure was implemented: initially, extracts were filtered through Whatman No. 1 filter paper for the removal of coarse particles and plant debris, followed by secondary filtration using 0.45 µm PTFE syringe filters (Millipore) for the elimination of fine particulates. The obtained clear supernatants were transferred to appropriate glass vials and stored at 4°C until phenolic and antioxidant analyses. This integrated extraction approach combines ultrasonic-assisted extraction, prolonged orbital shaking, and sequential filtration steps to achieve optimal transfer of target phytochemicals from dried plant material to the solvent phase.

For all antioxidant (DPPH, FRAP, CUPRAC) and colorimetric assays (TPC, TFC), extracts were dissolved in methanol to obtain a common stock solution. All assays were performed using this stock solution, followed by assay-specific dilutions within the linear measurement range. This approach ensured full comparability among methods.

2.3. Antioxidant activity assay

2.3.1. The chemicals

The following chemicals were acquired for the determination of total polyphenol, total flavonoids and antioxidant capacity: Methanol, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu's phenol reagent, and 2,2-diphenyl-1-picrylhydrazyl (DPPH), all of which were purchased from Sigma Chemical Co. in St. Louis, MO, USA. Additionally, sodium carbonate, acetic acid, neocuproine (2, 9-dimethyl-1, 10-phenanthroline), aluminum nitrate nonahydrate, and ammonium acetate were obtained from Merck Chemical Co. in Darmstadt, Germany. All of these chemicals used in the study were of analytical grade.

2.3.2. Determination of TPC

TPC was determined by the Folin–Ciocalteu colorimetric method [21]. An aliquot of each extract was diluted and mixed with Folin–Ciocalteu reagent and sodium carbonate, then incubated at room temperature. After the reaction, absorbance was measured at 760 nm. Gallic acid was used as the calibration standard, and TPC was expressed as mg gallic acid equivalents per gram of dry sample (mg GAE/g).

2.3.3. Determination of TFC

The TFC in the extracts was assessed through a colorimetric approach based on a modified procedure described by [22]. This method utilizes the ability of flavonoid molecules to form stable complexes with aluminum chloride ($AlCl_3$), specifically through interactions with the C-4 keto group and hydroxyl substituents at the C-3 or C-5 positions of flavones and flavonols. Moreover, it enables the formation of weaker complexes with ortho-dihydroxyl groups present on the A and B aromatic rings. Quercetin was employed as the reference standard across a concentration range of 0.03125 to 1.0 mg/mL. A calibration curve was constructed by plotting absorbance values against the respective quercetin concentrations, allowing the flavonoid content to be expressed as quercetin equivalents (QE).

2.3.4. DPPH radical scavenging assay

The capacity of extracts to neutralize DPPH radicals was evaluated using an adapted methodology based on Molyneux's protocol [23]. For this analysis, 0.75 mL of test samples prepared at different concentrations were mixed with equal volumes of DPPH solution (0.1 mM in methanol). These reaction systems were kept away from light at room temperature for 50 min to avoid photodegradation. Following incubation, spectrophotometric readings were taken at 517 nm

wavelength. Trolox served as the positive control for comparison. Extract effectiveness was determined as IC_{50} values (mg/mL), indicating the minimum concentration needed to neutralize half of the initial DPPH radicals, according to the calculations outlined by Gülçin and Alwasel [24].

2.3.5. FRAP assay

Extract samples were evaluated for their electron-donating abilities through the FRAP method, which measures conversion of ferric-tripyridyltriazine (Fe^{3+}-TPTZ) to its ferrous counterpart in acidic medium, producing an intense blue complex [25]. The protocol involved combining 100 µL of sample solution or solvent control with 3 mL of freshly made FRAP solution. Spectrophotometric measurements were recorded at 593 nm over 4 minutes while maintaining 25˚C. Quantification utilized a standard curve generated from $FeSO_4{\cdot}7H_2O$ solutions spanning 100-1000 µmol/L. Final data were reported as µmol Fe^{2+} equivalents per gram dried material.

2.3.6. CUPRAC assay

Extract samples underwent evaluation via the CUPRAC protocol, where antioxidants facilitate conversion of Cu(II)-neocuproine chelate to its Cu(I) state. This transformation produces a colored product with peak absorption at 450 nm. Standards were prepared using Trolox (vitamin E derivative) over concentrations from 0.03125-1 mM. Sample activities were quantified as TEAC values (Trolox Equivalent Antioxidant Capacity), providing normalized comparisons against the Trolox reference compound [26].

2.4. HPLC-DAD quantification of phenolic compounds

2.4.1. Chemicals and reagents

Acetonitrile (HPLC gradient) was obtained from Sigma-Aldrich Co. (St. Louis, MO, USA), and methanol (HPLC gradient) from Merck KGaA (Darmstadt, Germany). All phenolic standards were purchased from Sigma-Aldrich.

2.4.2. Chromatographic conditions

Phenolic compound analysis was performed using an Agilent 1260 Infinity II HPLC system with a diode array detector, following methods modified from [27,28]. Phenolic compounds in the flowers, leaves and steams of *Hypericum* species were determined using three analytical methods (Table 2):

(i) Method A: Separation of L-ascorbic acid, gallic acid, 3,4-dihydroxy benzoic acid, (+)-catechin, (-)-epicatechin, vanillic acid, rutin, p-coumaric acid, ferulic acid, rosmarinic acid and quercetin used an ACE 5 C18 column (250 × 4.6 mm, 5 µm). The mobile phase consisted of (A) acetonitrile and (B) 1.5% acetic acid solution, with a gradient: 15% A and 85% B initially, reaching 40% A and 60% B at 29 min. The system used a 1260 DAD WR detector (set at 250, 270, and 320 nm), 1260 Quaternary Pump (0.7 mL min^{-1}), a 1260 Vial Sampler (10 µL injection), and column oven at 35°C.

(ii) Method B: For pyrogallol, chlorogenic acid and syringic acid using the same column. The mobile phase: (A) methanol and (B) 1.5% acetic acid solution, with gradient: initially 10% A and 90 % B, 29 min 40% A and 60% B, 29-40 min 60% A and 40% B, 40-53 min 90% A and 10% B. Detection at 280, 290, 320, 370, and 535 nm, with same flow rate, injection volume, and temperature.

(iii) Method C: For hypericin, using the same C18 column. The mobile phase: (A) acetonitrile and (B) 20 mM ammonium acetate solution. The gradient was 50:50 (A:B) initially, then changed to 90:10 from 25 to 35 min, and returned to 50:50 from 35 to 45 min. The detector wavelengths were set at 590 nm and 600 nm. The flow rate was 1 mL min^{-1}, injection volume 50 µL, and column oven temperature 30 °C.

2.4.3. Preparation of standard solutions for phenolic compound quantification

Calibration curves used six concentrations of each phenolic standard (25, 50, 75, 100, 200, and 300 µg/mL). Standards were analyzed by HPLC-DAD to establish retention times and concentration-dependent absorbance.

Table 2. Proposed analytical method for determination of phenolic compounds in the flowers, leaves and steams of *Hypericum* species

Parameter	Method A	Method B	Method C
Column	ACE 5 C18 (250×4.6 mm, 5 µm)	ACE 5 C18 (250×4.6 mm, 5 µm)	ACE 5 C18 (250×4.6 mm, 5 µm)
Mobile Phase	(A) Acetonitrile, (B) 1.5% Acetic Acid	(A) Methanol, (B) 1.5% Acetic Acid	(A) Acetonitrile, (B) Ammonium Acetate
Gradient Program	0 min: 15% A, 85% B 29 min: 40% A, 60% B	0 min: 10% A, 90% B 29 min: 40% A, 60% B 29-40 min: 60% A, 40% B 40-53 min: 90% A, 10% B	0 min: 50% A, 50% B 25-35 min: 90% A, 10% B 35-45 min: 50% A, 50% B
Flow Rate	0.7 mL/min	0.7 mL/min	1 mL/min
Detection Wavelengths	250, 270, 320 nm	280, 290, 320, 370, 535 nm	595, 600 nm
Injection Volume	10 µL	10 µL	50 µL
Column Temperature	35 °C	35 °C	35 °C

2.4.4. Sample preparation and extraction

Extraction used a solvent system of acetonitrile and 1% acetic acid (9:1, v/v) as solution (a). A second mixture (b) combined solution (a) with methanol (1:1). Each dry plant sample (5 g) was extracted with 50 mL of the mixture via ultrasonication for 30 min, then incubated in darkness at room temperature for 24 h. Extracts were filtered before HPLC-DAD analysis.

2.5. Data analysis

All experiments were conducted using a completely randomized design. Data were analyzed by analysis of variance (ANOVA) followed by Duncan's multiple range test using SPSS 26 (IBM Corp., Armonk, NY, USA). All assays were performed in triplicate, and results are presented as mean ± standard error (SE). Differences were considered significant at $p < 0.05$.

3. Results and discussion

3.1. Total phenolic (TP) and total flavonoid (TF) contents

Phenolic and flavonoid contents exhibited substantial organ-specific variation in both species (Table 3).

Table 3. Total phenolics and flavonoid contents in the flower, leaf and stem of different *Hypericum* species.

Species	Parts	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg QE/g)
H. elongatum	Flower	21.52±0.9^{c}	7.99±0.4^{bc}
	Leaf	32.11±1.4^{b}	6.88±1.5^{c}
	Stem	7.63±0.2^{d}	1.89±0.2^{d}
H. orientale	Flower	37.87±1.9^{a}	10.66±0.3^{a}
	Leaf	30.68±0.8^{b}	9.31±1.2^{ab}
	Stem	22.74±1.1^{c}	2.49±0.2^{d}

In *H. elongatum*, leaves were predominant. Leaves contained the highest TPC (32.11±1.4 mg GAE/g). This value was 1.5-fold higher than flowers and 4.2-fold higher than stems. TFC showed a similar distribution pattern: leaves contained 6.88±1.5 mg QE/g, flowers 7.99±0.4 mg QE/g, and stems only 1.89±0.2 mg QE/g.

In *H. orientale*, flowers were dominant. The highest phenolic accumulation in this species was observed in flowers (37.87±1.9 mg GAE/g). Flowers contained 1.2-fold more phenolics than leaves and 1.7-fold more than stems. In terms of flavonoid content, flowers (10.66±0.3 mg QE/g) showed 4.3-fold higher values than stems.

These organ-specific differences stem from the two species following distinct adaptive strategies. The high phenolic levels in *H. elongatum* leaves indicate that the plant focuses on protecting its foliage. As photosynthetic organs, leaves are continuously exposed to UV radiation, drought, and herbivores. Phenolics protect these leaves both through their antioxidant effects and as UV shields [29]. This represents a "leaf defense" strategy.

H. orientale follows a different pathway: "reproductive defense". In this species, phenolics are concentrated in flowers. This has several rationales: flowers must be protected from UV damage, produce colored pigments to attract specific pollinators, and defend pollen and ovules against microbial attack [29,30]. The high flavonoid/phenolic ratio in *H. orientale* flowers (28.1%) supports this interpretation. Flavonoids are particularly important in floral pigmentation.

For broader perspective, comparison with other Hypericum species is necessary. *H. perforatum* typically contains 50–90 mg GAE/g TPC and 6–15 mg QE/g flavonoids [31,32]. Some species such as *H. montbretii* (90–100 mg GAE/g) and *H. scabrum* (>130 mg GAE/g) show considerably higher values [9,32].

However, these comparisons must be interpreted carefully. Multiple factors affect the results: which solvent was used (methanol or ethanol), extraction temperature and duration, when the plant was collected, at what altitude and under what conditions it grew, and how it was processed post-harvest. For instance, ethanol generally extracts more phenolics than methanol [33]. Plants grown at high altitudes also produce more phenolics because they are exposed to more UV radiation [32].

Despite these methodological differences, our findings are biologically meaningful. The values in our study (*H. orientale* flowers: 37.87 mg GAE/g, *H. elongatum* leaves: 32.11 mg GAE/g) are at levels sufficient for good antioxidant activity. The literature indicates that extracts with TPC >20 mg GAE/g show strong radical scavenging, while >30 mg GAE/g effectively prevents lipid oxidation [34]. Flavonoid levels >5 mg QE/g are also sufficient for anti-inflammatory and vasoprotective effects [33].

These findings are valuable for practical applications. Both species can be used as natural antioxidant sources. Potential exists for food preservation, cosmetic products, or dietary supplements. The substantial differences observed among organs necessitate changes in harvesting strategy. The traditional method uses the whole plant, but this is not logical. Stems contain very little phenolics and dilute the mixture.

In conclusion, our data demonstrate that selective harvesting targeting leaves for *H. elongatum* and flowers for *H. orientale* is much more efficient. This approach yields both more active compounds and higher quality extracts. This strategy is also consistent with modern practices recommended in medicinal plant cultivation and sustainable harvesting [35].

3.2. Antioxidant capacity: DPPH, FRAP, and CUPRAC assays

Antioxidant activity was evaluated using three complementary methods, revealing that organ-specific activity patterns are closely related to biochemical composition (Table 4). In *H. elongatum*, leaves exhibited dominant performance in all antioxidant tests. DPPH radical scavenging capacity was found to be extremely strong with IC_{50} = 0.02±0.001 mg/mL. The significance of this value is important: it means 5-fold more effective radical scavenging than flowers (0.10±0.01 mg/mL) and 3.5-fold more than stems (0.07±0.01 mg/mL). FRAP reducing power (299.38±14 µmol $FeSO_4{\cdot}7H_2O$/g) and CUPRAC chelating capacity (0.57±0.05 mmol TEAC/g) also confirmed the same hierarchy: leaves > stems ≈ flowers. This pattern is not coincidental. The antioxidant activity ranking shows perfect concordance with the previously determined TPC and TFC distribution (Table 3). The strong relationship between high phenolic content (32.11 mg GAE/g) and potent antioxidant capacity is clearly evident. This finding points to two critical conclusions: First, phenolics in *H. elongatum* leaves are not only abundant in quantity but also present in biochemically active forms. Second, the "quantity = quality" equation is valid in this species, meaning phenolic richness directly translates into antioxidant potential.

An interesting divergence is observed in *H. orientale*. All three organs showed similar scavenging capacity in the DPPH test (IC_{50}= 0.04–0.06 mg/mL), meaning they are equivalent in terms of radical scavenging. However, an unexpected result emerged in the FRAP and CUPRAC tests: stems exhibited approximately 1.3-fold higher reducing power than flowers and leaves (FRAP= 191.81±6 µmol, CUPRAC= 0.40±0.04 mmol). This finding appears paradoxical at first glance because flowers had the highest total phenolic content (37.87 mg GAE/g). This discrepancy demonstrates that antioxidant activity depends more on specific phenolic composition than total phenolic quantity. FRAP and CUPRAC tests specifically measure metal chelation and electron transfer capacity, and this activity depends on the presence of specific phenolic types [36]. For example, compounds containing multiple hydroxyl groups such as pyrogallol can show much stronger metal reduction capacity than flavonols at the same concentration [37]. Therefore, the FRAP/CUPRAC superiority of *H. orientale* stems originates from selective accumulation of phenolic types with particularly strong reducing capacity in this tissue.

When we evaluate our findings in the context of the broader *Hypericum* genus, both of our species show performance consistent and competitive with the literature. *H. elongatum* leaves possess radical scavenging capacity equivalent to the reference species *H. perforatum* (IC_{50} = 0.02–0.05 mg/mL) [38,39]. Similarly, they are at comparable levels with *H. scabrum* (IC_{50} = 0.03–0.06 mg/mL) [40]. In terms of FRAP values (299 µmol), they fall in the same category as *H. perforatum* methanolic extracts (200–350 µmol) [39]. This similarity is important because *H. perforatum* is a species with clinically proven antioxidant activity, used on a commercial scale. The fact that *H. elongatum* leaves show the same performance as this species is a concrete indicator of its pharmaceutical potential.

A similar situation exists for *H. orientale*. DPPH (IC_{50} = 0.04–0.06 mg/mL) and FRAP values (135–192 µmol) are in the same range as *H. triquetrifolium* (IC_{50} = 0.039–0.062 mg/mL) [41] and *H. cordifolium* leaf extracts (IC_{50} = 0.061 mg/mL) [42], both Mediterranean and Asian species with established antioxidant properties. These systematic similarities are not coincidental. Species-level antioxidant capacity fundamentally reflects the evolutionary conservation of secondary metabolite biosynthesis pathways. The *Hypericum* genus is rich in flavonoids and phenolic acids, in addition to characteristic naphthodianthrones such as hypericin and hyperforin, and phloroglucinol derivatives. Therefore, the activity similarity among different species is a reflection of a shared biochemical heritage. In conclusion, both of our species possess potential comparable to known antioxidant-rich species in the *Hypericum* genus. Particularly, the low IC_{50} value (0.02 mg/mL) shown by *H. elongatum* leaves indicates that this species can be evaluated as a scientifically valid and competitive natural antioxidant source for nutraceutical, cosmeceutical, or food preservation applications. Additionally, the FRAP/CUPRAC superiority in *H. orientale* stems is also valuable from a practical perspective. Stem tissue is generally considered harvest waste, but our findings show that this tissue can be a valuable resource particularly for applications requiring metal chelation and lipid oxidation prevention.

Table 4. Contents of FRAP, CUPRAC and DPPH in the flower, leaf and stem of different *Hypericum* species.

Species	Parts	DPPH IC_{50} (mg/ml)	FRAP (µmol $FeSO_4.7H_2O$/g)	CUPRAC (mmol TEAC/g)
H. elongatum	Flower	0.10±0.01^{b}	112.98±10.7^{d}	0.06±0.01^{e}
	Leaf	0.02±0.001^{a}	299.38±14^{a}	0.57±0.05^{a}
	Stem	0.07±0.01^{b}	25.95±1.2^{e}	0.09±0.01^{d}
H. orientale	Flower	0.06±0.001^{b}	143.14±9^{c}	0.33±0.07^{c}
	Leaf	0.06±0.01^{b}	135.14±6.7^{c}	0.35±0.01^{c}
	Stem	0.06±0.01^{b}	191.81±6^{b}	0.40±0.04^{b}
Standard	—	0.0037	—	—

3.3. Phenolic compound profiles

As stated in the Materials and Methods section, Method A, Method B, and Method C were used in the combined analysis of 15 phenolic and flavonoid compounds. The HPLC profile preserved the same elution sequence, and the elution times for each peak are shown in Fig. 2, Fig 3, and Fig. 4 (see Table 5). HPLC-DAD analysis revealed distinct organ-specific metabolic specialization patterns in both species (Table 6).

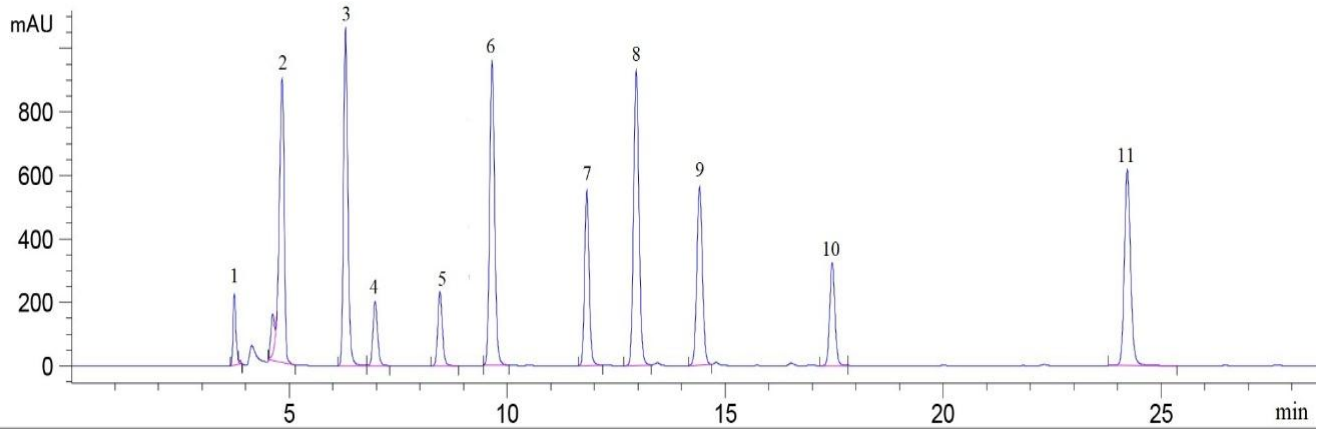

Figure 2. The HPLC profile of the phenolics using method A. Their symbols and retention times are as follows: ascorbic acid (AsA), 3.74(1); gallic acid, 4.84(2); 3,4-dihydroxybenzoic acid, 6.29(3); catechin, 6.96(4); epicatechin, 8.45(5); vanillic acid, 9.65(6); rutin, 11.81(7); p-coumaric acid, 12.95(8); ferulic acid, 14.41(9); rosmarinic acid, 17.44(10); quercetin, 24.2(11).

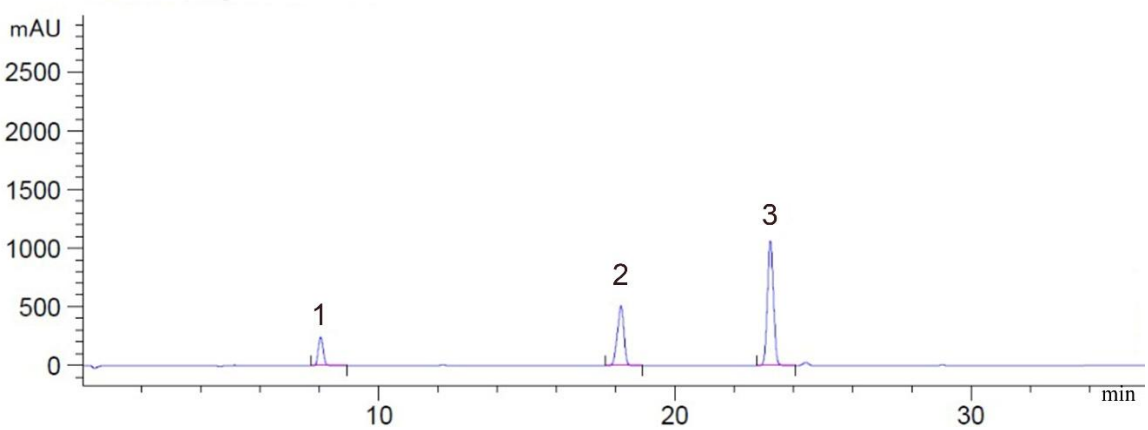

Figure 3. The HPLC profile of the phenolics using method B. Their symbols and retention times are as follows: pyrogallol, 8.045(1); chloragenic acid, 18.184(2); syringic acid, 23.228(3)

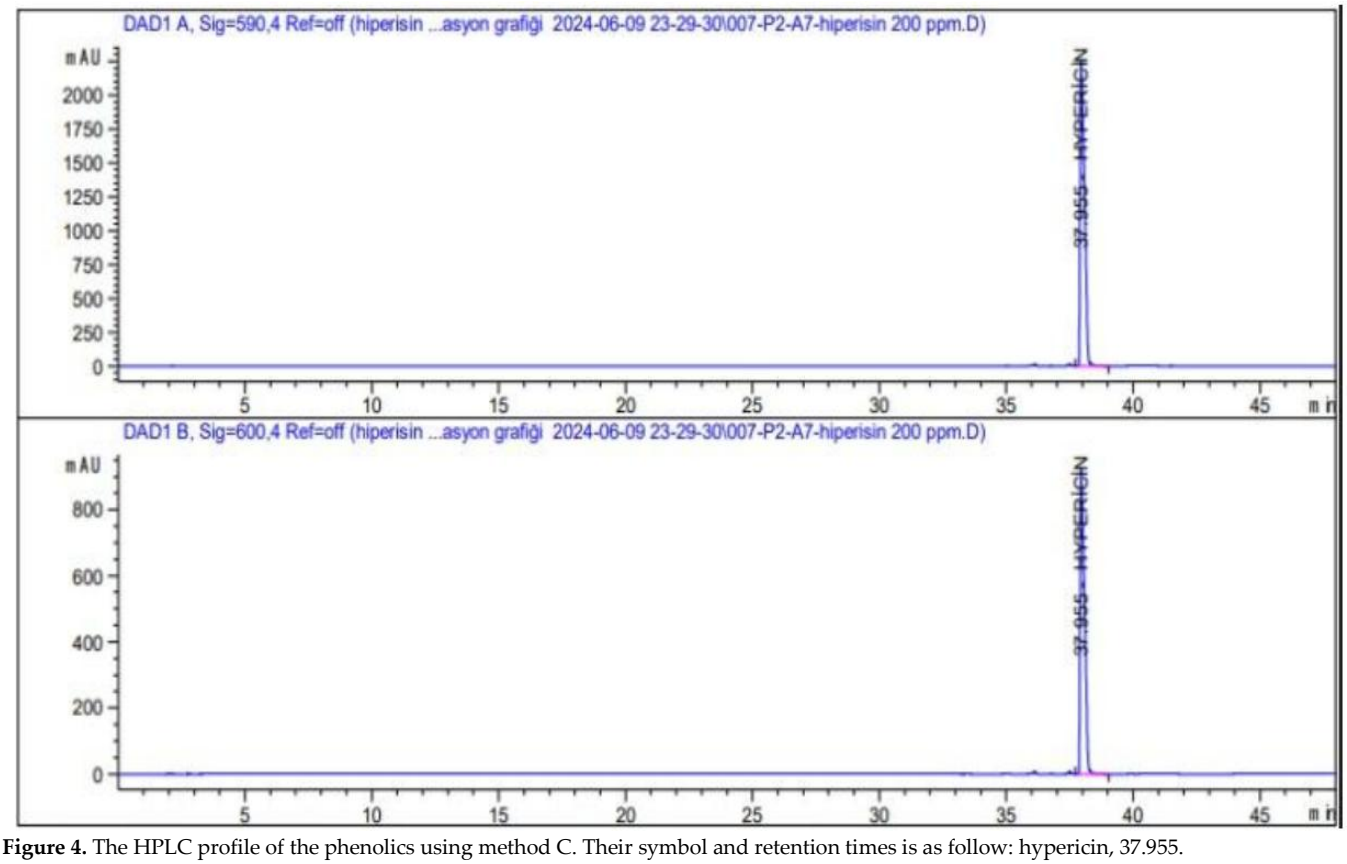

Figure 4. The HPLC profile of the phenolics using method C. Their symbol and retention times is as follow: hypericin, 37.955.

Table 5. Repeat injection counts and R^2 correlation coefficients of calibration curves for standard chemical compounds used in the HPLC analysis

No	Standard Chemical Compound Names	Repeat Injection Count	R^2 Value
1	L-Ascorbic acid	3	0.9972
2	Gallic acid	3	0.9999
3	3,4-Dihydroxy benzoic acid	3	0.9997
4	(+)-Catechin	3	0.9991
5	(-)-Epicatechin	3	0.9980
6	Vanillic acid	3	0.9988
7	Rutin	3	0.9997
8	p-Coumarıc Acid	3	0.9988
9	Ferulic acid	3	0.9976
10	Rosmarinic acid	3	0.9996
11	Qercetin	3	0.9966
12	Pyrogallol	3	0.9950
13	Chloragenic acid	3	0.9973
14	Syringic acid	3	0.9994
15	Hypericin	3	0.9987

Table 6. Comparative phenolic compounds (mg/kg) in methanolic extracts of flower, leaf, and stem from different *Hypericum* species

	H. elongatum			*H. orientale*		
Phenolic Compounds	**Flower**	**Leaf**	**Stem**	**Flower**	**Leaf**	**Stem**
Ascorbic acid	1256	258.7	172.6	987.9	826.3	N/A
Gallic acid	123.9	N/A	N/A	426.1	605.8	N/A
3,4 hydroxy benzoic acid	N/A	N/A	N/A	1237.9	657.84	79.65
Catechin	1225.1	1769.2	534.9	N/A	1438.8	644.2
Epicatechin	1864.3	2586.9	838.7	433.8	2630.2	1410.6
Vanillic acid	3168.6	311.6	0.7	71.8	1938.7	10.29
Rutin	223.2	26.2	10.9	N/A	57.36	6.9
p-coumaric acid	529.5	619.9	151.1	68.2	1584.2	N/A
Ferulic acid	N/A	253.3	41.1	275.7	1327.3	N/A
Rosmarinic acid	7.1	28.1	8.1	11.6	30.3	7.14
Quercetin	344.5	177.6	105.9	1314.2	476.96	31.5
Pyrogallol	129.93	975.27	112.53	638.19	913.74	705.45
Chloragenic acid	68.2	148.6	24.56	156.28	434.26	41.25
Syringic acid	N/A	1.72	N/A	12.53	14.18	6.53
Hypericin	19.03	15.6	4.51	27.5	16.06	13.52

H. elongatum exhibits a strong antioxidant defense strategy in primary photosynthetic tissues. Leaves, as tissues with intense reactive oxygen species (ROS) production due to continuous light exposure and high photosynthetic activity, accumulate flavan-3-ols extensively: catechin (1769.2 mg/kg) and epicatechin (2586.9 mg/kg). This accumulation is accompanied by hydroxycinnamic acids: p-coumaric acid (619.9 mg/kg), ferulic acid (253.3 mg/kg), and chlorogenic acid (148.6 mg/kg). This phenolic profile indicates that leaves invest metabolically in constitutive defense pathways to protect the photosynthetic apparatus from oxidative damage. Flowers exhibit a different physiological prioritization. The accumulation of high vanillic acid (3168.6 mg/kg) and ascorbic acid (1256.0 mg/kg) reflects protective mechanisms of reproductive organs against developmental stress. While moderate flavan-3-ols are maintained (catechin 1225.1 mg/kg, epicatechin 1864.3 mg/kg), the increase in flavonol glycosides (quercetin 344.5 mg/kg, rutin 223.2 mg/kg) supports UV-B filtering and pollinator signaling functions. Stems, as metabolically less active support tissues, show lower phenolic investment: epicatechin (838.7 mg/kg), catechin (534.9 mg/kg), and minimal pyrogallol (112.53 mg/kg).

Organ-specific metabolic differentiation is much more pronounced in *H. orientale*. Leaves exhibit exceptional phenolic diversity: epicatechin (2630.2 mg/kg), p-coumaric acid (1584.2 mg/kg), vanillic acid (1938.7 mg/kg), catechin (1438.8 mg/kg), ferulic acid (1327.3 mg/kg), gallic acid (605.8 mg/kg), 3,4-dihydroxybenzoic acid (657.84 mg/kg), chlorogenic acid (434.26 mg/kg), quercetin (476.96 mg/kg), and ascorbic acid (826.3 mg/kg). This broad-spectrum phenolic repertoire provides flexible defense capacity against multiple stress factors (UV radiation, herbivory, pathogen attack). Flowers display a distinct metabolic profile focused on optimizing reproductive success.

The dominance of quercetin (1314.2 mg/kg) reflects a powerful UV-protective and antioxidant function that enhances pollen viability and fertilization success. The abundance of 3,4-dihydroxybenzoic acid (1237.9 mg/kg), ascorbic acid (987.9 mg/kg), and gallic acid (426.1 mg/kg) forms an antimicrobial and antioxidant shield for developing seeds. A striking metabolic distinction: catechin was completely suppressed in *H. orientale* flowers (not detected), whereas in *H. elongatum* it was present at 1225.1 mg/kg. This indicates that the species regulate flavan-3-ol biosynthetic pathways in a tissue-specific manner.

A physiologically important metabolic pattern is observed in stems. The most abundant phenolic is epicatechin (1410.6 mg/kg), followed by pyrogallol (705.45 mg/kg) and catechin (644.2 mg/kg). Pyrogallol (1,2,3-trihydroxybenzene) is rarely found in free form in plants and typically arises from gallotannin hydrolysis during extraction [43]. Under methanolic extraction, galloyl ester bonds break, gallic acid is released and undergoes decarboxylation to yield pyrogallol [34]. This presence of pyrogallol at this level in *H. orientale* stems indicates abundant gallotannin reserves serving structural and defensive functions [44]. Stem woody tissues accumulate tannins in cell walls to provide long-lived mechanical support and protect the vascular system from pathogens. The lower pyrogallol levels in *H. elongatum* stems (112.53 mg/kg) indicate fundamental differences between species in tannin-based defense strategies. Although pyrogallol is likely extraction-derived, both gallotannins and their hydrolysis products are biologically active: they exhibit antioxidant, antimicrobial, and anti-inflammatory properties [45].

Hypericin, a naphthodianthrone-class secondary metabolite that is an adaptive feature of the *Hypericum* genus, varies in distribution between species (Table 6).

H. elongatum shows flower-focused accumulation: 19.03 mg/kg (flower), 15.6 mg/kg (leaf), 4.51 mg/kg (stem). *H. orientale* exhibits more homogeneous distribution: 27.5 mg/kg (flower), 16.06 mg/kg (leaf), 13.52 mg/kg (stem). Hypericin in *H. orientale* stems is three-fold higher than in *H. elongatum* stems, indicating that this species maintains photoactive defense mechanisms even in stem tissues. Concentrations (0.045–0.275%) fall within the reported range (0.009–0.512%) [46,10] and are consistent with *H. perforatum* (0.1–0.5% in flowers) [12]. Hypericin's antidepressant, antimicrobial, photodynamic, and antitumor properties [46] highlight the species' pharmacological potential.

The relationship between phenolic composition and antioxidant capacity confirms organ-specific physiological strategies. Metabolic consistency is observed in *H. elongatum*: TPC, individual phenolics, and all antioxidant tests show the same hierarchy (leaf > flower > stem). The exceptional activity of leaves (DPPH IC_{50} = 0.02 mg/mL, FRAP = 299 μmol, CUPRAC = 0.57 mmol) is directly attributable to intensive flavan-3-ol accumulation (catechin + epicatechin = 4356 mg/kg, approximately 42% of total phenolics). Flavan-3-ols are potent antioxidants due to catechol and hydroxyl groups that protect photosynthetic membranes from lipid peroxidation, scavenge chloroplast ROS, and stabilize photoinhibited PSII [37,48]. This supports the photoprotective mechanisms of leaves under high light stress.

The metabolic-activity relationship in *H. orientale* is more complex and reflects tissue-specific functional priorities. TPC ranks as flower > leaf > stem, but FRAP/CUPRAC show stem > flower ≈ leaf, and DPPH remains homogeneous (IC_{50} = 0.04–0.06 mg/mL). This apparent contradiction is explained by the structural efficiency of stem phenolics. Although pyrogallol comprises only 3.1% of total stem phenolics (0.705 mg/g within 22.74 mg GAE/g total), its three ortho-hydroxyl groups create extraordinary electron donor capacity and show 5–10-fold superior performance in metal reduction tests compared to catechin/gallic acid [49,50]. This structural feature indicates that gallotannin "derived compounds" in stems are optimized for protecting vascular tissues against metal-catalyzed oxidative stress through efficient electron transfer mechanisms. Importantly, pyrogallol's advantage is specific to electron-transfer mechanisms; the advantage is less pronounced in hydrogen-donation-based DPPH tests [51]. Flowers and leaves, rich in quercetin (1314.2 mg/kg) and epicatechin (2630.2 mg/kg), provide balanced, multi-mechanistic antioxidant defense.

These findings confirm established ecological-physiological strategies in the *Hypericum* genus. *H. perforatum* shows similar tissue-specific metabolic specialization: flavan-3-ol dominance in leaves (photoprotection), hypericin/flavonol richness in flowers (UV defense, pollinator relationships) [47,38]. Flavan-3-ol accumulation in leaves also supports inducible defense responses. Fungal infection triggers catechin synthesis and inhibits pathogen growth through antimicrobial activity [52]. Flavonols in flowers absorb UV-B at 280–320 nm, protecting pollen and ovules from DNA damage [29]. Ascorbic acid abundance supports the ascorbate-glutathione cycle for oxidative protection of developing embryos [53]. Comparison with other Mediterranean species (*H. triquetrifolium*, *H. cordifolium*) reveals genus-level conserved metabolic programs: flavan-3-ols, flavonols (quercetin, rutin), and hydroxycinnamic acids (chlorogenic, p-coumaric) form recurrent patterns across species [42]. This metabolic conservatism reflects successful adaptations of the *Hypericum* lineage to shared environmental pressures (high light, UV-B, biotic stress).

When all these findings are integrated, it emerges that *H. elongatum* and *H. orientale* follow different but complementary metabolic strategies. *H. elongatum* exhibits a photosynthetic tissue-centered defense approach: high total phenolic content (32.11 mg GAE/g), dominant flavan-3-ol profile (catechin + epicatechin = 4356 mg/kg), and strong antioxidant activity directly correlated with these (DPPH IC_{50} = 0.02 mg/mL, FRAP = 299 μmol). This pattern reflects a strategy that invests metabolically in constitutive antioxidant defense in photosynthetic tissues facing continuous light exposure and high ROS production. *H. orientale* shows more complex tissue-specific metabolic differentiation: high total phenolics (37.87 mg GAE/g) and quercetin/hypericin richness in flowers optimizing reproductive success, moderate pyrogallol (705 mg/kg) in stems providing structurally efficient reducing power despite lower total phenolic content. These findings demonstrate that antioxidant potential depends not only on total phenolic quantity but also on the structural properties and tissue-specific physiological functions of specific compounds (flavan-3-ols, quercetin, pyrogallol). The fact that both species show antioxidant activity comparable to *H. perforatum*, *H. triquetrifolium*, and other Mediterranean species confirms conserved secondary metabolite pathways and shared ecological adaptations in the *Hypericum* genus. The pronounced differences between organ-specific phenolic composition and antioxidant capacity reveal the critical importance of organ selection for herbal applications. *H. elongatum* leaves and *H. orientale* flowers can be evaluated as scientifically valid natural antioxidant sources for food supplements, cosmetic products, and food preservation applications.

4. Conclusion

This study demonstrates that phenolic composition and antioxidant capacity are organ-specific in the flowers, leaves, and stems of *H. elongatum* and *H. orientale*. *H. elongatum* leaves exhibit high flavan-3-ol content (catechin and epicatechin) with strong radical scavenging and reducing capacity, while *H. orientale* flowers are rich in quercetin and hypericin. A notable finding is that *H. orientale* stems display exceptional reducing power (FRAP and CUPRAC) despite the lowest total phenolic content, explained by the presence of pyrogallol (705 mg/kg), a gallotannin hydrolysis product with superior electron-donating capacity due to its three ortho-hydroxyl groups. This demonstrates that antioxidant potential depends not only on total phenolic quantity but also on the identity and structural properties of specific compounds.

Strong correlations were observed between TPC, TFC, and antioxidant capacity, confirming that these metabolites play a central role in the redox-regulatory potential of both species. Both species demonstrate antioxidant performance comparable to the clinically validated *H. perforatum*, positioning them as underutilized candidates for phytotherapeutic development. These results emphasize the importance of selecting specific plant organs rather than using the whole plant when developing phytotherapeutic or nutraceutical preparations from *Hypericum* species.

Future studies should proceed in three main directions. First, the bioavailability and extent of absorption of these compounds (catechin, epicatechin, quercetin, hypericin) in the body must be investigated. Second, whether the strong antioxidant activity observed in the laboratory actually translates into anti-inflammatory, antimicrobial, wound-healing, or neuroprotective effects in living systems must be tested. Third, the biological significance of extraction-derived pyrogallol and the actual activity of gallotannin precursors should be evaluated. These investigations will determine whether in vitro activities translate into functional therapeutic outcomes and will support the development of effective phytotherapeutic applications from these species.

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