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Comparative analysis of phenolic profiles and antioxidant capacities in the flowers, leaves, and stems of *Hypericum montanum* L.

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Abstract

This study presents the first comprehensive organ-based analysis in the literature that the quantitative distribution of 25 phenolic compounds in the flower, leaf, and stem methanolic extracts of *Hypericum montanum* L. was evaluated and supported by antioxidant assays. Total phenolic and flavonoid contents were determined for each organ, and antioxidant capacities were assessed using DPPH, FRAP, and CUPRAC methods. The results revealed notable chemical differences among the plant organs.

In the flower, high levels of flavonoids such as epicatechin, catechin, hesperetin, and quercetin were detected, which was consistent with the strong antioxidant activity observed in the CUPRAC and DPPH assays. Phenolic acids such as rosmarinic acid and 3,4-dihydroxybenzoic acid, which were abundant in the leaf, were consistent with high FRAP values. These findings suggest that certain plant organs may possess potential for inclusion in natural antioxidant-enriched formulations.

Additionally, the phenolic composition data may offer preliminary insights into the chemotaxonomic differentiation of *H. montanum* from morphologically similar species. In this respect, the study provides supplementary evidence that could contribute to future taxonomic evaluations. Overall, the findings lay a foundation for more detailed investigations into the phytochemical and biological properties of *H. montanum.*

Keywords: *Hypericum*, antioxidant activity, HPLC analysis, phenolic compounds, flavonoids, chemotaxonomy

1. Introduction

The genus *Hypericum* L. (Hypericaceae) is a large taxonomic group represented by approximately 500 species worldwide [1]. Most members of this genus are herbaceous plants, although some may occasionally attain shrubby or small tree forms. Characteristic morphological features include pentamerous, actinomorphic flowers, typically yellow petals, numerous stamens, and distinctive secretory structures in leaves and flowers that are taxonomically informative [2]. In the flora of Türkiye, *Hypericum* is represented by over 100 taxa, more than 60 of which are endemic [3,4]. Given this richness, Türkiye is considered one of the most important centers for *Hypericum* diversity and endemism in Europe and Western Asia. This notable taxonomic diversity also reflects the significant pharmaceutical and ethnobotanical potential of the genus.

Hypericum species have long been utilized in traditional medicine for their wound-healing, anti-inflammatory, antidepressant, and antimicrobial properties [5,6]. Among these, *H. perforatum* L. (commonly known as St. John's Wort) is widely used in various formulations such as ointments, teas, and tinctures. In modern phytotherapy, extracts of this species are commercially available in Europe as over-the-counter herbal remedies, particularly in the treatment of mild to moderate depression [7]. In addition, some *Hypericum* species have attracted attention for their antioxidant capacity, phenolic compound profiles, and cytotoxic effects, indicating a potential use as functional food ingredients or natural preservatives [8,9]. Despite its broad geographical range, available data on the phytochemical profiles and biological activities of underexplored species such as *H. montanum* remain limited. Therefore, comprehensive phytochemical and biological evaluations of such species are of considerable importance, both for their potential traditional use and their unexplored chemical diversity.

Over the past two decades, research efforts have increasingly focused on the remarkable phenolic

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diversity and biological potential of the *Hypericum* genus. In this context, species such as *H. perforatum*, *H. scabrum*, *H. origanifolium*, *H. montbretii*, and *H. orientale* have been studied in terms of total phenolic and flavonoid content, antioxidant capacity (typically through DPPH, FRAP, or ABTS assays), volatile compound profiles, and quantitative analyses of selected phenolic constituents [10–13]. However, a substantial proportion of these studies have either been limited to a single plant organ (often the whole aerial part or leaves) or have focused on a narrow set of phenolic compounds. Furthermore, variations in extraction solvents, analytical techniques, and the selection of reference compounds have limited the comparability of results across studies.

Despite its natural distribution in Europe and Western Asia, *H. montanum* remains relatively underexplored compared to more commonly studied congeners. Existing literature on this species has primarily addressed antimicrobial effects or general antioxidant activity. To date, no comprehensive HPLC-based quantitative evaluation of its organ-specific phenolic composition appears to have been published. In the present study, methanolic extracts derived from the flowers, leaves, and stems of *H. montanum* were analyzed for total phenolic and flavonoid content, and antioxidant potential was assessed using DPPH, FRAP, and CUPRAC assays. In addition, the organ-specific quantitative distribution of 25 selected phenolic compounds was determined using HPLC. To the best of the authors' knowledge, this study represents the first example in the literature to present such an extensive, multifaceted, and organ-based phytochemical investigation of *H. montanum*.

This study aims to establish, to our knowledge for the first time, a quantitative organ-specific HPLC profile of 25 phenolic compounds in the flowers, leaves, and stems of *Hypericum montanum* L., and to determine the correlation between phytochemical diversity and biological activity by linking these profiles to DPPH, FRAP, and CUPRAC antioxidant assay results. The high-quality reference data generated will serve as a resource for natural product development and chemotaxonomic differentiation of morphologically similar species.

2. Experimental

2.1. Plant collection, identification, and voucher information

The plant material of *H. montanum* examined in this study was collected from its natural habitat in the Kafkasör region of Artvin, Türkiye (1060 m a.s.l.) on 23 June 2022. During collection, morphological features potentially important for taxonomic identification, as well as ecological and habitat-related observations, were carefully documented. Photographs were taken in situ to support identification (Fig. 1). The taxonomic identification and verification of the species were carried out in accordance with the keys provided by Robson [14] and Ekim [15]. The collected specimen has been deposited under the accession number "Aksu 370" in the Herbarium of the Medicinal and Aromatic Plants Application and Research Centre at Artvin Çoruh University.

2.2. Extraction protocol

The collected *H. montanum* specimens were air-dried under shaded conditions for approximately 5-7 days. After drying, the plant material was manually separated into flowers, leaves, and stems, each of which was then ground into a fine powder using a laboratory-grade blender. For extraction, a measured amount of each powdered organ was immersed in methanol and agitated at 25 °C for 24 hours using a mechanical shaker. The mixtures were subsequently filtered through Whatman filter paper, and the filtrates were concentrated under reduced pressure via rotary evaporation. The resulting methanolic extracts were subjected to a series of antioxidant analyses, including total phenolic and total flavonoid content determination, as well as DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (Ferric Reducing Antioxidant Power), and CUPRAC (Cupric Ion Reducing Antioxidant Capacity) assays. In addition, high-performance liquid chromatography (HPLC) was employed to characterize the phenolic composition of the extracts in detail.

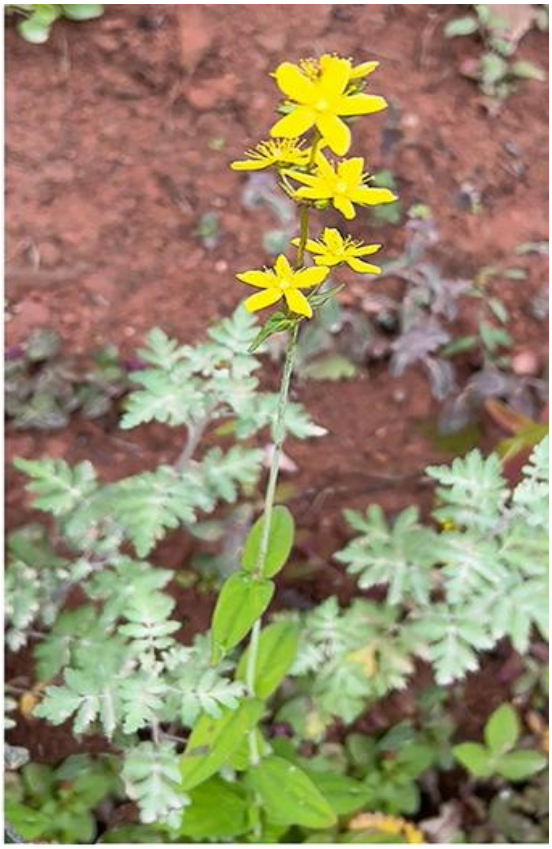

Figure 1. Habit of *H. montanum* in the field

2.3. Determination of total phenolic content via folin–ciocalteu assay

The total phenolic content of the extracts was quantified using the Folin–Ciocalteu colorimetric assay. In this method, phenolic compounds present in the sample reduce the Folin–Ciocalteu reagent, resulting in the formation of a blue-colored complex with a characteristic absorbance at 760 nm. The intensity of the developed color is directly proportional to the concentration of phenolic constituents in the extract. A standard calibration curve was constructed using known concentrations of gallic acid, and the results were expressed as milligrams of gallic acid equivalents (mg GAE) per gram of extract, following the procedure described by Singleton et al. [16].

2.4. Determination of total flavonoid content using aluminum chloride complexation

The total flavonoid concentration in the extracts was assessed through a colorimetric approach based on a modified procedure described by [17]. 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).

2.5. DPPH radical scavenging assay

The antioxidant capacity of the extracts against the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was determined following a modified protocol adapted from Molyneux (2004) [18]. A 0.75 mL aliquot of each sample at various concentrations was combined with 0.75 mL of a 0.1 mM methanolic DPPH solution. The reaction mixtures were allowed to incubate in the dark at ambient temperature for 50 minutes to prevent light-induced degradation. Subsequently, the decrease in absorbance was measured at 517 nm using a UV-Vis spectrophotometer. Trolox was employed as the reference antioxidant compound. The DPPH scavenging efficiency of each extract was expressed as IC_{50} (mg/mL), representing the concentration of sample required to reduce the initial DPPH concentration by 50%, as described by Gülçin and Alwasel (2023) [19].

2.6. Assessment of antioxidant potential via the FRAP method

The ferric reducing antioxidant power (FRAP) assay was used to assess the total antioxidant potential of the extracts, based on the ability of antioxidants to reduce the Fe^{3+}-TPTZ (2,4,6-tripyridyl-s-triazine) complex to its Fe^{2+} form under acidic conditions, resulting in a blue-colored solution [20]. In this procedure, 3 mL of freshly prepared FRAP reagent was mixed with 100 µL of either the test extract or extraction solvent (blank) in a test tube. The absorbance was monitored at 593 nm for a duration of 4 minutes at a constant temperature of 25 °C. A calibration curve constructed using $FeSO_4{\cdot}7H_2O$ in the range of 100 to 1000 µmol/L was used to quantify the reducing capacity. The results were expressed as micromoles of Fe^{2+} equivalents per gram of dry sample.

2.7. Determination of antioxidant activity using the CUPRAC assay

The antioxidant potential of the extracts was assessed using the CUPRAC (Cupric Ion Reducing Antioxidant Capacity) method, which is based on the reduction of the copper(II)-neocuproine [Cu(II)-Nc] complex to the copper(I)-neocuproine [Cu(I)-Nc] form in the presence of antioxidant compounds. This redox reaction leads to the generation of a chromogenic complex that exhibits maximum absorbance at 450 nm. For calibration, Trolox—a water-soluble analogue of vitamin E—was used as the reference standard across a concentration range of 0.03125 to 1 mM. The antioxidant activity of each sample was calculated and expressed as Trolox Equivalent Antioxidant Capacity (TEAC), enabling a standardized comparison of antioxidant effectiveness relative to Trolox [21].

2.8. High-performance liquid chromatography conditions for phenolic compound identification

High-performance liquid chromatography (HPLC) analysis was conducted using an ACE 5 C18 column (250 × 4.6 mm, i.d.) to achieve separation of phenolic constituents. In the first method, the mobile phase consisted of solvent A (acetonitrile) and solvent B (1.5% acetic acid in water). The gradient elution was programmed to start at 15% A and 85% B, gradually shifting to 40% A and 60% B over a 29-minute period. The chromatographic system included a 1260 DAD WR detector set to monitor at 250, 270, and 320 nm wavelengths, along with a 1260 Quaternary Pump delivering a flow rate of 0.7 mL/min, a 1260 Vialsampler set to inject 10 µL of sample, and a G7116A column compartment maintained at 35 °C.

For the secondary HPLC procedure, the same ACE 5 C18 column was employed. Here, the mobile phase consisted of solvent A (methanol) and solvent B (1.5%

Table 1. Chromatographic parameters applied in the HPLC System

Parameter	Method A	Method B
Analyzed Compounds	13 standard phenolic compounds	10 standard phenolic compounds
Chromatographic System	HPLC-DAD (High-Performance Liquid Chromatography – Diode Array Detector)	HPLC-DAD (High-Performance Liquid Chromatography – Diode Array Detector)
Column	ACE 5 C18 (250 * 4.6 mm, 5 µm)	ACE 5 C18 (250 * 4.6 mm, 5 µm)
Mobile Phase	Gradient phase of acetonitrile (A) and 1.5% acetic acid (B)	Gradient phase of methanol (A) and 1.5% acetic acid (B)
Run Time	29 minutes	53 minutes
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
Retention Time Range	~3.74 min (Ascorbic acid) – ~29.08 min (Apigenin)	~8.04 min (Pyrogallol) – ~52.93 min (Chrysin)
Injection Mode	Individual and combined injections	Individual and combined injections
Injection Volume	10 µL	10 µL
Column Temperature	35 °C	35 °C
Purpose	Separation and quantification of common phenolic acids and flavonoids	Identification of additional phenolics like stilbenes and flavones
Elution Consistency	Consistent elution order in single and mixed injections	Consistent elution order in single and mixed injections

acetic acid aqueous solution). The gradient program started at 10% A and 90% B, increased to 40% A and 60% B by the 29th minute, then to 60% A and 40% B from 29 to 40 minutes, and finally to 90% A and 10% B between 40 and 53 minutes. Detection was carried out using a 1260 DAD WR detector configured at multiple wavelengths (280, 290, 320, 370, and 535 nm), supported by the same 1260 Quaternary Pump (flow rate: 0.7 mL/min), 1260 Vialsampler (10 µL injection volume), and thermostated column oven set to 35 °C. The chromatographic conditions applied in both HPLC methods are summarized in Table 1 for clarity and comparison purposes.

The chromatographic separation of standard phenolic compounds was visualized through HPLC chromatograms to ensure peak identification and retention time consistency. These are illustrated in Fig. 2, Fig. 3, and Fig. 4.

2.9. Statistical processing of antioxidant data

All antioxidant assays, including total phenolic content (TPC), total flavonoid content (TFC), DPPH radical scavenging activity (IC_{50}), FRAP, and CUPRAC analyses, were performed in triplicate. The obtained data were subjected to statistical evaluation using one-way analysis of variance (ANOVA) with IBM SPSS Statistics software (version 27). Mean comparisons between plant organs were conducted using Duncan's multiple range test. A p-value of less than 0.05 was considered statistically significant.

3. Results and dicussion

3.1. Total phenolic content (TPC)

In this study, total phenolic content (TPC) levels were determined in the flower, leaf, and stem organs of *H. montanum*, and statistically significant differences were

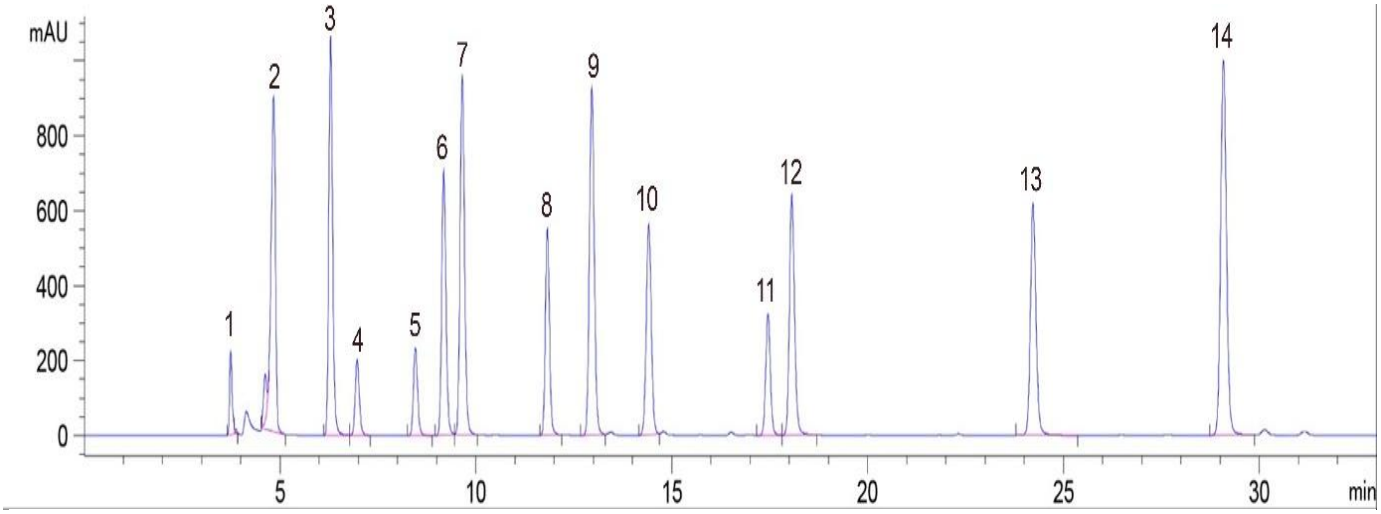

Figure 2. Representative HPLC chromatogram of phenolic standards with corresponding retention times: ascorbic acid (AsA) at 3.74 min (1), gallic acid at 4.84 min (2), 3,4-dihydroxybenzoic acid at 6.29 min (3), catechin at 6.96 min (4), epicatechin at 8.45 min (5), caffeic acid at 9.17 min (6), vanillic acid at 9.65 min (7), rutin at 11.81 min (8), p-coumaric acid at 12.95 min (9), ferulic acid at 14.41 min (10), rosmarinic acid at 17.44 min (11), myricetin at 18.04 min (12), quercetin at 24.20 min (13), and apigenin at 29.08 min (14)

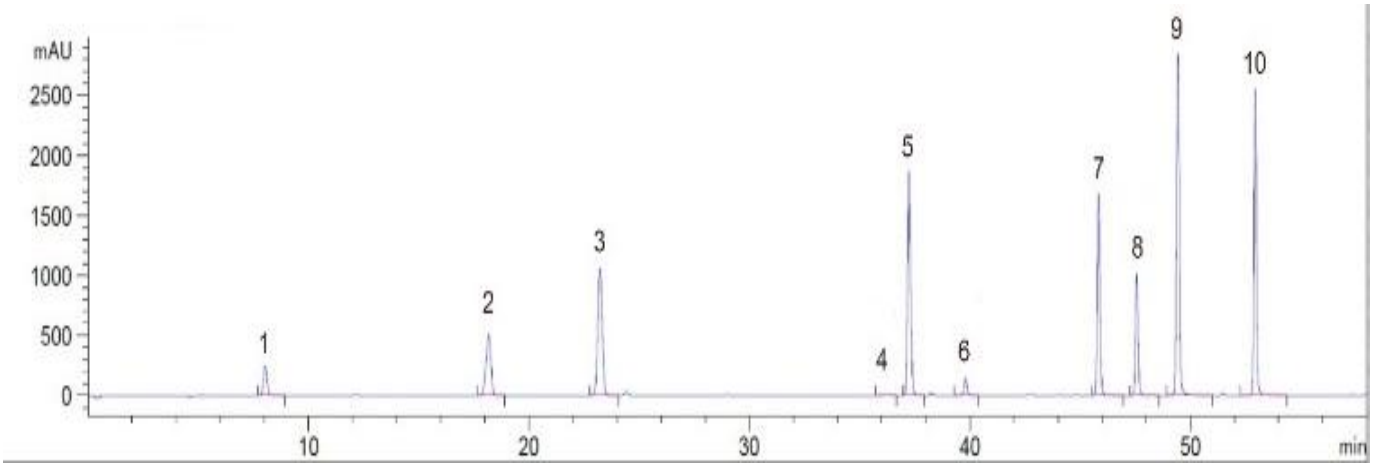

Figure 3. HPLC chromatogram displaying retention times of selected phenolic standards: progallol at 8.045 min (1), chlorogenic acid at 18.184 min (2), syringic acid at 23.228 min (3), cyanidin chloride at 36.248 min (4), resveratrol at 37.236 min (5), oleuropein at 39.810 min (6), hesperetin at 45.84 min (7), kaempferol at 47.562 min (8), baicalin at 49.435 min (9), and chrysin at 52.939 min (10)

observed among the organs. The highest phenolic content was detected in the leaf (70.68 ± 1.19 mg GAE/g), followed by the flower (68.61 ± 1.73 mg GAE/g), and the lowest was found in the stem (31.85 ± 0.76 mg GAE/g) (Table 2).

These findings reveal that the distribution of phenolic compounds within the species varies substantially among plant organs. Leaves typically contain higher levels of phenolics due to the greater abundance of secretory structures (such as translucent and black glands), which serve as major sites of polyphenol biosynthesis and accumulation [12,22,23]. In addition, leaves are exposed to environmental stressors (e.g., UV radiation, wind, pathogens) for longer periods compared to flowers, which may lead to enhanced secondary metabolite production and phenolic accumulation [24,25].

Similar trends have been reported in other *Hypericum* species, such as *H. perforatum*, *H. montbretii*, and *H. orientale*, in which leaf extracts consistently exhibit higher phenolic content than those from flower or stem [11,12]. For instance, phenolic concentrations in the leaves of *H. montbretii* have been reported to range between 336.91 and 394.30 mg GAE/g, whereas stem values are markedly lower.

Moreover, environmental conditions such as altitude, light intensity, temperature, and nutrient availability can significantly affect phenolic biosynthesis. Leaf phenolic accumulation often increases under stress conditions, including higher altitudes, elevated UV exposure, and lower temperatures [26,27]. However, in some cases, the opposite trend has been observed, depending on the interplay between species-specific traits and environmental variables [28,29].

The TPC values determined for *H. montanum* in this study are comparable to those reported for methanolic extracts of *H. salsolifolium* (68.9 ± 3.44 mg GAE/g), and similar levels were observed in water extracts (64.52 ± 3.22 mg GAE/g) [30]. In *H. scabrum*, TPC values were recorded as 76.27 ± 2.59 mg GAE/g in flower and 94.85 ± 0.07 mg GAE/g in the aqueous residue of aerial parts [23, 31]. Conversely, ethanol–water extracts of *H. perforatum* flowers showed substantially higher TPC values: 371 ± 49 mg GAE/g in air-dried samples and 238 ± 26 mg GAE/g in lyophilized material [32]. Additionally, studies across various *Hypericum* taxa have reported TPC values in methanolic extracts ranging from 64.08 to 505.7 mg GAE/g, highlighting both methodological and species-specific biochemical variability [33].

Overall, the TPC level of *H. montanum* is comparable to that of related species when methanol or water is used as the solvent. However, it remains lower than the richer phenolic profiles obtained through ethanol–water extractions. This observation underscores the significant influence of extraction medium, plant organ, and processing conditions on phenolic yield. Moreover, variation in phenolic content across plant organs enables more precise analysis of organ-specific biological activities and phytochemical evaluations. By presenting organ-based quantitative data for a relatively understudied species, this study makes a meaningful contribution to the understanding of the chemical diversity of *H. montanum.*

In conclusion, the high phenolic concentrations observed in the leaves and flowers of *H. montanum* can be explained by both anatomical features and environmental influences. The relatively low phenolic content in the stem may be attributed to the limited presence of phenolic-producing structures in this organ. To the best of our knowledge, this is the first study to evaluate organ-specific phenolic content in *H. montanum* in such detail, providing valuable insight into the intraspecific chemical variability of the species.

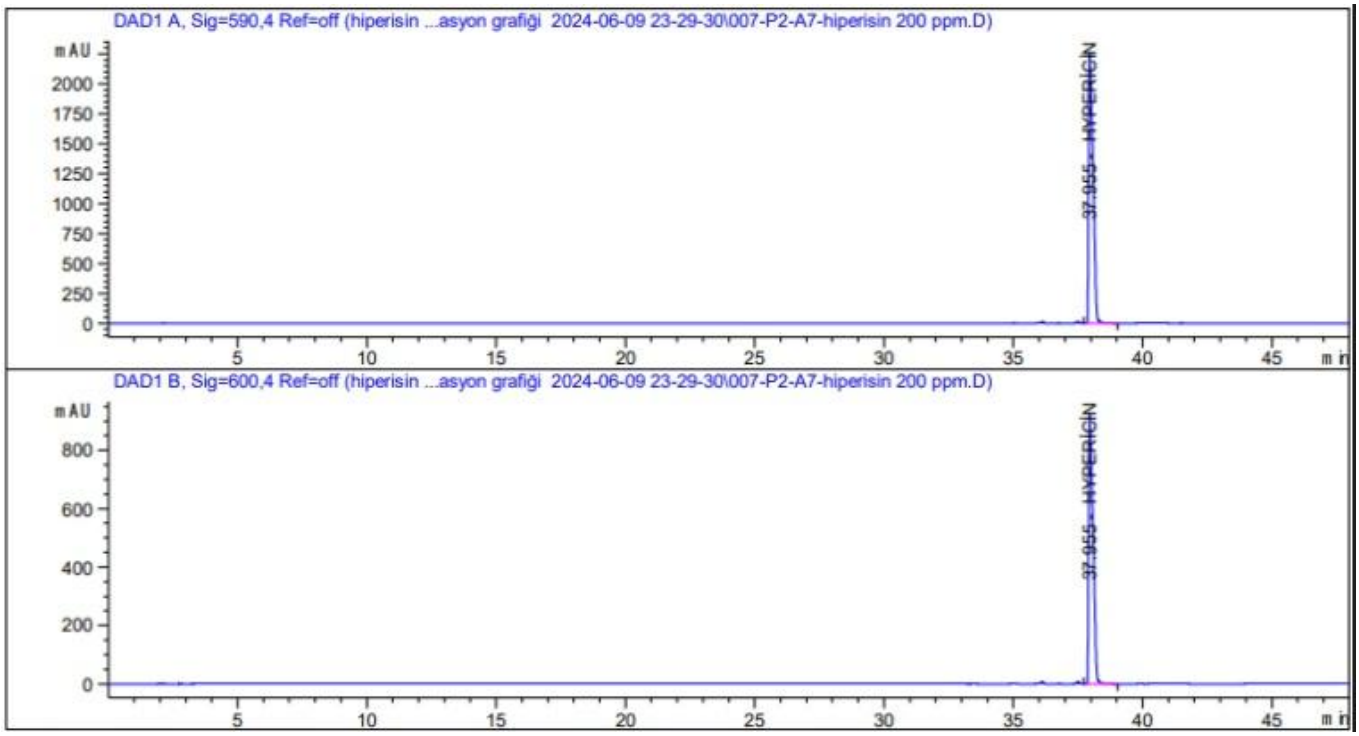

Figure 4. HPLC chromatogram illustrating the retention time of the hypericin standard.

3.2. Total flavonoid content (TFC)

In this study, the total flavonoid contents of the flower, leaf, and stem of *H. montanum* were determined, and notable differences were observed among the plant parts.

The highest flavonoid concentration was found in the flowers (32.11 ± 0.68 mg QE/g), followed by the leaves (21.32 ± 0.42 mg QE/g), and the lowest in the stems (4.34 ± 0.24 mg QE/g) (Table 2).

The organ-specific distribution of flavonoids is closely associated with the density and type of cellular structures in which these compounds are synthesized and stored. Flowers are primary sites for the biosynthesis of pigment-related flavonoids, particularly flavonol glycosides and anthocyanins, and therefore may exhibit a distribution pattern distinct from that of general phenolics [13,34,35]. In fact, previous studies have shown that flowers of species such as *H. perforatum* and *H. scabrum* contain higher levels of flavonoids than their respective leaves [12,36,37].

The elevated levels of flavonoids in flowers may also be linked to their protective role against environmental stress factors such as UV-B radiation and temperature fluctuations [27,38]. Certain flavonoids present in flowers are known to possess strong UV-absorbing capacities, which may contribute to their preferential accumulation in these organs. Although substantial flavonoid content is also observed in leaves, factors such as interspecific variation and growth stage can influence their levels [11,39].

Comparative literature data further contextualize the flavonoid richness of *H. montanum*. For instance, methanolic extracts of endemic *H. salsolifolium* flowers were reported to contain 104.69 ± 5.23 mg QE/g, markedly higher than those of *H. montanum*, underscoring its exceptional flavonoid biosynthetic capacity [30]. Likewise, *H. heterophyllum* leaf extracts yielded 45.2 ± 1.3 mg QE/g under similar methanolic conditions, suggesting that *H. montanum* flowers possess a moderate yet noteworthy flavonoid profile in comparison to related taxa [40]. In *H. perforatum*, a well-documented species, air-dried flower extracts obtained using ethanol reached up to 160 mg CAE/g depending on harvest time, emphasizing the impact of both solvent polarity and phenological timing on flavonoid yield [32].

Table 2. Comparative antioxidant evaluation of flower, leaf, and stem extracts of *Hypericum montanum*

Taxon	Used part	Total phenolic content (mg GAE/g dry sample)*	Total flavonoid content (mg QE/g dry sample)*	IC_{50} DPPH Inhibition (mg/ml)*	FRAP (µmol $FeSO_4.7H_2O$/g sample)*	CUPRAC (mmol TEAC/g sample)*
H. montanum	Flower	68.61±1.73^{ab}	32.11±0.68^{a}	0.01±0.00^{c}	96.96±17.17^{c}	1.30±0.10^{a}
	Leaf	70.68±1.19^{a}	21.32±0.42^{b}	0.03±0.01^{b}	288.83±6.20^{a}	0.91±0.05^{b}
	Stem	31.85±0.76^{c}	4.34±0.24^{c}	0.07±0.0^{a}	164.83±21.27^{b}	0.34±0.04^{c}
Standard	—	—	—	0.0037±0.0	—	—

Standard: IC_{50} value of the reference antioxidant (Trolox) calculated from the standard calibration curve. Values are expressed as mean ± SD (n=3). Statistical significance was determined by one-way ANOVA followed by Duncan's test. Differences were considered significant at $p < 0.05$.

In the case of *H. montanum*, available literature on flavonoid content is generally limited, and most studies have focused on whole plant extracts without organ-specific separation. To the best of the author's knowledge, this is the first study to report the total flavonoid content of *H. montanum* in a quantitative and organ-specific manner based on methanolic extracts. In this regard, the present work fills a significant gap in the literature and provides valuable insights into the organ-level distribution of bioactive compounds related to the species' potential pharmacological activity.

3.3. DPPH free radical scavenging capacity (IC_{50})

In this study, the DPPH radical scavenging capacities of *H. montanum* extracts from different plant organs were evaluated based on IC_{50} (mg/mL) values. The lowest IC_{50} value was observed in the floral extract (0.01 ± 0.00 mg/mL), indicating the strongest antioxidant activity. The leaf extract showed a moderate activity with an IC_{50} of 0.03 ± 0.01 mg/mL, while the stem extract exhibited the weakest effect with an IC_{50} of 0.07 ± 0.01 mg/mL (Table 2).

The DPPH assay reflects the capacity of antioxidant compounds to donate hydrogen atoms or electrons to neutralize DPPH free radicals [41]. This mechanism is particularly relevant to hydroxyl-rich flavonoids, which effectively reduce DPPH radicals. The high antioxidant activity observed in the floral extract is in line with its flavonoid content (32.11 mg QE/g). Moreover, the IC_{50} value of 0.01 mg/mL obtained in our study is higher than that of the synthetic antioxidant Trolox (0.0037 mg/mL) under comparable conditions. Nevertheless, this result still indicates a substantial radical-scavenging capacity for a natural extract, highlighting the potent antioxidant potential of the flowers.

Similar patterns have been reported in *Hypericum perforatum*, *H. montbretii*, and *H. orientale*, where flower extracts demonstrated higher DPPH activity than those obtained from leaves and stems [11,36]. The results for leaf and stem extracts in our study were consistent with their respective total flavonoid and phenolic content, showing moderate and low activity, respectively. Comparative studies provide additional context for these findings. For example, Kakouri et al. (2023) [42] reported floral IC_{50} values ranging from 0.01045 mg/mL (*H. perforatum*) to 0.02839 mg/mL (*H. vesiculosum*) across nine Greek *Hypericum* species, placing *H. montanum* at the high-performance end of the spectrum. Likewise, IC_{50} values reported for leaf (≈0.02–0.05 mg/mL) and stem (≥0.05 mg/mL) extracts in related species align closely with those observed in our study [43]. This consistency supports the view that *H. montanum* flowers exhibit a particularly robust DPPH-scavenging potential comparable to, or even exceeding, many well-studied congeners.

To the best of our knowledge, this is the first study to report IC_{50} values for *H. montanum* methanolic extracts in an organ-specific manner using the DPPH assay. These findings contribute significantly to filling a gap in the literature regarding the antioxidant properties of this species.

In conclusion, the antioxidant activities of organ-specific methanolic extracts of *H. montanum* were systematically evaluated using the DPPH assay. The floral extract demonstrated particularly strong free radical scavenging potential, suggesting that flowers may serve as a primary biological source for further pharmacological evaluation of this species.

3.4. Ferric reducing antioxidant power (FRAP)

In this study, the FRAP values of flower, leaf, and stem extracts of *H. montanum* were determined as 96.96 ± 17.17, 288.83 ± 6.20, and 164.83 ± 21.27 μmol $FeSO_4{\cdot}7H_2O$/g, respectively. The highest ferric reducing power was observed in the leaf extract, followed by the stem and flower extracts (Table 2).

The FRAP method evaluates antioxidant potential based on the reducing capacity of the extract and is particularly sensitive to the presence of polyphenolic compounds [20]. The high FRAP value observed in the leaf extract in this study is consistent with its elevated total phenolic content (70.68 mg GAE/g).

In the literature, variable FRAP values have been reported for different organs of *Hypericum* species. For instance, the stem extract of *H. perforatum* was reported to have a FRAP value of 235.90 μmol Fe^{2+}/g [36], while the leaf and flower extracts of *H. scabrum* were reported as 115.99 and 34.09 μmol Fe^{2+}/g, respectively [44]. In comparison, the FRAP value measured in the leaf extract of *H. montanum* (288.83 μmol Fe^{2+}/g) is higher than those reported for many *Hypericum* species.

Agan et al. (2023) [30], reported a FRAP value of 308.21 ± 15.41 mg Trolox/g for the methanolic extract of endemic *H. salsolifolium*, which is comparable to that of *H. montanum* leaves. Similarly, Zhang et al. (2023) [45], found a FRAP value of 1.22 ± 0.086 mmol Fe^{2+}/L in hydroalcoholic extracts of *H. attenuatum*. Babota et al. (2021) [46], measured 2.66 ± 0.031 and 2.58 ± 0.036 mM Fe^{2+} for *H. spectabile* and *H. thymbrifolium*, respectively. These values suggest that *H. montanum* demonstrates a relatively high ferric-reducing antioxidant potential within the genus.

A positive relationship between FRAP values and total phenolic content has frequently been reported in the literature. In this context, the high FRAP value observed in the leaf extract of our study may be attributed to the parallel trend with phenolic content. On

the other hand, no similarly strong correlation was observed between flavonoid content and FRAP value. This may suggest that the contribution of flavonoid compounds to ferric reducing capacity is more limited compared to phenolics and may depend on the structural properties and types of individual flavonoids.

The FRAP results support the association between total antioxidant capacity and the presence of phenolic compounds. It has also been noted that FRAP values can vary significantly depending on the extraction method, solvent used, and environmental factors [47]. In this regard, the similarity of the FRAP data obtained in our study with values reported for other *Hypericum* species supports the methodological consistency of the present work.

In conclusion, the leaf extract of *H. montanum* exhibited the highest ferric reducing power among all tested organs, and this finding demonstrated a meaningful parallelism with its total phenolic content. This result suggests that the leaf of *H. montanum*, due to its rich phenolic profile and ferric reducing capacity, may be considered among plant materials with natural antioxidant properties. However, confirmation of this potential requires further support through different extraction techniques, in vivo biological assessments, and broader-spectrum antioxidant assays..

3.5. CUPRAC (cupric ion reducing antioxidant capacity)

In this study, the CUPRAC values of *H. montanum* extracts obtained from flower, leaf, and stem were determined as 1.30 ± 0.10, 0.91 ± 0.05, and 0.34 ± 0.04 mmol TEAC/g, respectively. The highest CUPRAC value was observed in the floral extract, which corresponded with its high flavonoid content (32.11 mg QE/g) (Table 2).

The CUPRAC method evaluates the antioxidant capacity of compounds based on their ability to reduce Cu^{2+} ions to Cu^{+} and allows the simultaneous assessment of both hydrophilic and lipophilic antioxidants [21]. Compared to other assays such as DPPH and FRAP, it provides a broader-spectrum evaluation. Previous studies have reported that CUPRAC values in *H. perforatum* and *H. scabrum* are positively correlated with flavonoid content [10,48,49]. In support of these findings, recent studies have also documented notable CUPRAC differences among *Hypericum* species and organs. For instance, *H. perforatum* leaf extracts prepared with methanol exhibited a CUPRAC value of 3307.84 ± 45.87 µmol TE/mL, substantially higher than that of its floral counterparts [50]. Similarly, water and hydroalcoholic extracts from *H. perforatum* flowers were found to have lower CUPRAC capacities than ethanol-extracted leaves, suggesting a strong influence of flavonoid concentration and extraction efficiency [51]. These observations align with the high CUPRAC value recorded in the floral extract of *H. montanum* (1.30 ± 0.10 mmol TEAC/g), which likely reflects the abundance of flavonoids in the flower. Since CUPRAC selectively responds to hydroxyl-rich flavonoids, a positive correlation between CUPRAC response and flavonoid content has also been consistently demonstrated [21,52].

In our study, the CUPRAC value of the floral extract was found to be significantly higher than those of the leaf and stem extracts ($p < 0.05$). This suggests that the CUPRAC method may be particularly sensitive to flavonoid-type compounds. The lower CUPRAC activity in the leaf and stem extracts may be associated with their relatively lower flavonoid content.

Nevertheless, the observation that the highest antioxidant activity was recorded in the leaf extract via the FRAP assay, and in the floral extract via the CUPRAC assay, indicates that antioxidant capacity can vary depending on the assay employed. Since each method demonstrates selective sensitivity to specific groups of compounds, the use of multiple complementary assays is recommended for a more comprehensive evaluation of antioxidant potential in plant extracts [52,53].

In conclusion, the floral extract of *H. montanum* exhibited the highest antioxidant capacity in the CUPRAC assay. This finding suggests that the flower of *H. montanum*, due to its rich flavonoid content and copper ion reducing potential, may represent a promising natural source of antioxidants. However, this potential should be further confirmed through additional extraction protocols, in vivo biological assays, and broader-spectrum antioxidant evaluations..

3.6. Interpretation of HPLC findings and organ-specific distribution of phenolic compounds

In this study, the quantitative distribution of 25 phenolic compounds in the flower, leaf, and stem organs of *H. montanum* was analyzed using HPLC based on methanolic extracts. The data revealed distinct differences in compound profiles among plant organs (Table 3).

According to the HPLC analysis results, the most abundant flavonoid compounds in the floral extract of *H. montanum* were epicatechin (5948.92 mg/L), catechin (4758.84 mg/L), hesperetin (2846.97 mg/L), quercetin (1828.53 mg/L), and chrysin (278.88 mg/L), respectively (Table 3). Among these flavonoids, epicatechin and catechin are notable for their strong antioxidant activity, as well as their neuroprotective and cardiovascular-supportive effects [54,55]. These two compounds have also been previously detected in high amounts in the flowers and leaves of species such as *H. perforatum*; for

example, catechin and epicatechin levels in *H. perforatum* have been reported as 5257.2 mg/L and 6396.94 mg/L, respectively [10,56]. The similar concentrations measured in *H. montanum* suggest that this species may share a comparable phenolic profile with other members of the genus, indicating intraspecific chemical continuity. Hesperetin belongs to the flavanone group and exhibits anti-inflammatory, antioxidative, and vascular-supportive properties [57,58]. Although hesperetin is rarely detected in *Hypericum* species in the literature, its high concentration observed in this study (2846.97 mg/L) is noteworthy. Quercetin, on the other hand, is known for its anti-inflammatory, antiproliferative, and hepatoprotective effects and has been reported as a major bioactive compound in many *Hypericum* species [10,45,56,59].

Another prominent flavonoid in the flower is chrysin, a flavone that is particularly known for its anti-inflammatory, antiproliferative, and antioxidant properties. Chrysin's ability to modulate oxidative stress responses and inhibit certain enzymatic systems makes it a pharmacologically attractive compound [45,59]. The level of chrysin detected in *H. montanum* suggests that the biological effects associated with antioxidant activity may arise from a multifaceted compound interaction.

In terms of phenolic compounds, the floral extract was richest in oleuropein (4853.97 mg/L), 3,4-dihydroxybenzoic acid (1995.6 mg/L), progallol (1971.75 mg/L), and coumaric acid (1647.9 mg/L) (Table 3). Oleuropein is a compound commonly found in olive leaves and is widely studied for its strong antioxidant, antimicrobial, and neuroprotective properties [60]. While high levels of oleuropein are rarely reported in the *Hypericum* genus, its presence in both the stem and the floral extract of *H. montanum* at such concentrations may be considered characteristic of this species. 3,4-dihydroxybenzoic acid is known for its protective effects against reactive oxygen species and is an important phenolic acid involved in antioxidant defense mechanisms [61]. Progallol, a simple phenolic compound containing three hydroxyl groups, is recognized for its hydroxyl radical scavenging capacity and its role in phenolic antioxidant systems [49,62].

Most of these compounds have also been identified in similar forms in other *Hypericum* species, particularly

Table 3. Comparative phenolic composition of flower, leaf, and stem of *Hypericum montanum* methanolic extract

No	Compounds	*H. montanum*		
		Flower (mg/L)	Leaf (mg/L)	Stem (mg/L)
		Vitamin C		
1	Ascorbic acid	516.54	500.6	324.22
		Phenolics		
2	Gallic acid	N/D	N/D	213.54
3	3,4 hydroxy benzoic acid	1995.6	48.2	N/D
4	Vanillic acid	95.68	50.9	28.68
5	Syringic acid	N/D	N/D	N/D
6	Coumaric acid	1647.9	1893.16	N/D
7	Caffeic acid	N/D	N/D	41.81
8	Ferulic acid	591.71	438.68	N/D
9	Rosmarinic acid	90.22	37.53	14.11
10	Progallol	1971.75	N/D	28.5
11	Chlorogenic acid	170.38	N/D	76.63
12	Resveratrol	10.63	N/D	N/D
13	Oleuropein	4853.97	N/D	181.75
	Total Phenolics	**11427.84**	**2468.47**	**909.24**
		Flavonoids		
14	Catechin	4758.84	1821.65	1305.45
15	Epicatechin	5948.92	3633.1	1046.9
16	Rutin	80.35	103.65	7.05
17	Myricetin	23.22	N/D	N/D
18	Quercetin	1828.53	265.72	38.09
19	Apigenin	N/D	N/D	36.13
20	Cyanidin chloride	N/D	N/D	N/D
21	Hesperitin	2846.97	N/D	N/D
22	Kaempferol	N/D	N/D	N/D
23	Baicalin	21.08	N/D	15.05
24	Chrysin	278.88	N/D	N/D
	Total Flavanoids	**15786.79**	**5824.12**	**2448.67**
		Naphthodianthrone		
25	Hypericin	14.35	188.97	13.39

N/D: Not Detected

H. perforatum, *H. montbretii*, and *H. scabrum*, where they have been reported at high concentrations [10,49,56,62]. For instance, in *H. perforatum*, catechin, epicatechin, and quercetin have been reported at levels of 5257.2 mg/L, 6396.94 mg/L, and 1227.6 mg/L, respectively. Although compounds such as hesperetin and chrysin have also been identified in *H. montbretii* and *H. scabrum*, the levels vary significantly across species [45,59,63]. In contrast, the hesperetin and oleuropein levels detected in the flowers of *H. montanum* in this study were found to be higher than those reported for many other *Hypericum* species, highlighting the species' distinct phenolic profile. Recent comparative HPLC-based studies have also documented significant variations in phenolic compound accumulation across different *Hypericum* species and organs. For example, the levels of catechin (4758.84 mg/L) and epicatechin (5948.92 mg/L) identified as dominant flavonoids in the flowers of *H. montanum* align with previous reports from *H. perforatum* leaves (5257.2 and 6396.94 mg/L, respectively) [64]. Another notable flavonoid, chrysin, was found at a concentration of 278.88 mg/L in *H. montanum*, while *H. xylosteifolium* reported substantially lower levels (37.22 mg/L) [65], supporting the organ- and species-specific nature of phenolic distribution. Additionally, Alahmad et al. (2022) [66], demonstrated that among five *Hypericum* species examined (*H. perforatum*, *H. maculatum*, *H. hirsutum*, *H. montanum*, and *H. tetrapterum*), *H. montanum* exhibited the highest levels of hypericin and pseudohypericin, emphasizing its pharmacological relevance. Strong correlations between hypericin and pseudohypericin contents further reinforce the biosynthetic linkage and chemotaxonomic relevance of these compounds. In contrast, quercetin and quercitrin levels were comparatively lower in *H. montanum* than in *H. perforatum* and *H. maculatum*, reflecting species-specific flavonoid biosynthetic differences [65,66]. These findings support the organ-specific distribution patterns observed in this study and reinforce the utility of HPLC profiling in the chemotaxonomic and pharmacological characterization of *Hypericum* species.

These differences may be attributed to environmental factors such as habitat conditions, altitude, light, and soil properties at the sampling location, as well as to genetic variation and intraspecific differences [65,67]. Therefore, the phenolic compounds detected at high levels in *H. montanum* may not only reflect the species' pharmacological potential but also serve as distinctive chemotaxonomic markers. In addition, the hypericin concentrations detected in the analyzed organs are noteworthy. In particular, the level measured in the leaf (188.97 mg/L) was considerably higher than those found in the flower (14.35 mg/L) and stem (13.39 mg/L). Hypericin is a naphthodianthrone derivative specific to *Hypericum* species and is considered a key secondary metabolite due to its potential photodynamic, antidepressant, antiviral, and antitumor properties [5,67].

In the literature, especially in *H. perforatum*, hypericin has been reported to accumulate in the leaf and flower in association with black-secreting glands [3,67]. The substantially higher concentration observed in the leaf of *H. montanum* compared to the flower and stem may similarly suggest a localization of hypericin in regions rich in these glandular structures. Moreover, since the distribution of hypericin among species is influenced by both environmental and genetic factors [65], the elevated levels found in the leaves of *H. montanum* may represent not only a pharmacologically relevant trait, but also a potentially distinct chemotaxonomic marker for the species. Although it has been reported to exhibit morphological similarity to *H. bithynicum* [49], the two species show notable differences in their phenolic compound profiles. Specifically, *H. bithynicum* has been shown to contain lower levels of catechin and epicatechin in flowers compared to *H. montanum*, and differences have also been noted in the accumulation patterns of chlorogenic and neochlorogenic acids [49]. While the chemotaxonomic interpretation of *Hypericum* species remains complex due to intraspecific and environmental variability, previous research has indicated that phenolic compound profiles—particularly flavonoids and phenolic acids—may serve as supplementary data in distinguishing morphologically similar taxa [63]. In this context, the distinct phenolic fingerprint of *H. montanum* identified in this study could contribute valuable biochemical evidence to future multidimensional taxonomic evaluations involving morphological, molecular, and phytochemical approaches.

Additionally, a notable strength of this study lies in the organ-specific approach used for the HPLC analysis. To the best of the authors' knowledge, this is the first study to systematically quantify 25 phenolic compounds in three distinct plant parts (flower, leaf, and stem) of *H. montanum*. Thus, the study contributes not only to elucidating intraspecific chemical variability but also to addressing the gap in the literature regarding organ-specific phenolic profiling of this species.

The findings suggest that the flower, due to its high levels of flavonoids (such as epicatechin, catechin, hesperetin, and quercetin) with known antioxidant, anti-inflammatory, and cytoprotective effects, may have potential application in phytotherapeutic preparations rich in natural antioxidants. The substantial presence of phenolic acids such as rosmarinic acid and 3,4-dihydroxybenzoic acid in the leaves, consistent with their ferric reducing capacity, indicates that the leaf may

be suitable for the development of antioxidant-focused biological products. The detection of compounds such as oleuropein and chlorogenic acid in the stem suggests potential for antimicrobial or metabolic regulatory uses. However, further confirmation through bioavailability studies, toxicological evaluations, and in vivo pharmacological modeling is necessary to substantiate these prospects.

Moreover, the organ-specific HPLC data obtained in this study showed a general correlation with the results of the total phenolic content (TPC), total flavonoid content (TFC), and antioxidant activity assays (DPPH, FRAP, CUPRAC). Notably, the high concentrations of flavonoids in the flower were consistent with the strong antioxidant activity measured in the CUPRAC and DPPH assays, while the abundance of phenolic acids in the leaves was reflected in the high TPC and FRAP values. Similar relationships between compound classes and antioxidant assay mechanisms have been documented in the literature, where phenolic acids are typically more active in ferric reduction, whereas flavonoids contribute more effectively to radical scavenging and copper ion reduction [10,56,63].

In this context, the data obtained not only present a quantitative assessment of phenolic and flavonoid content but also provide a more holistic evaluation of the phytochemical and biological potential of *H. montanum* through the integration of organ-specific antioxidant capacity analyses.

4. Conclusions

This study represents the first comprehensive organ-based analysis in the literature in which a total of 25 phenolic compounds were quantitatively evaluated in methanolic extracts and supported by various antioxidant tests. Thanks to the determination of phenolic and flavonoid contents for each organ, along with commonly used antioxidant assays such as DPPH, FRAP, and CUPRAC, meaningful connections were established between the plant's phytochemical profile and its biological activity.

In particular, the high antioxidant capacity observed in the CUPRAC and DPPH analyses was consistent with the elevated levels of flavonoid compounds such as epicatechin, catechin, hesperetin, and quercetin detected in the flower; whereas phenolic acids such as rosmarinic acid and 3,4-dihydroxybenzoic acid found predominantly in the leaf showed a parallel trend with the FRAP data. These compounds have been reported in the literature to possess antioxidant, cardiovascular-supporting, anti-inflammatory, and cytoprotective effects, and are evaluated in various pharmaceutical and functional product formulations.

In this context, certain compounds identified in specific organs of *H. montanum* may be considered noteworthy at the component level in research aimed at developing natural antioxidant-based products. For example, flavan-3-ol derivatives such as epicatechin and catechin are investigated in cardiovascular support formulations; flavonoids like hesperetin and quercetin in anti-inflammatory systems; and rosmarinic acid as a stabilizing antioxidant in topical and food-supported products. This chemical composition supports the possibility that *H. montanum* may be among species with versatile biological potential.

Although species such as *H. perforatum*, which have been more extensively studied in the literature, are widely used in traditional medicine, studies on *H. montanum* remain quite limited. This study reveals the chemical composition of the species in detail, providing a foundation for both scientific and application-oriented evaluations. The findings suggest that this species may be a potential candidate for evaluation in phytotherapeutic research, particularly in the development of antioxidant-focused formulations. Furthermore, the phenolic profile obtained in this study offers data that may allow for comparative evaluation of *H. montanum*'s chemical composition with that of morphologically similar species, and in this respect, may contribute to examining potential chemotaxonomic distinctions.

However, for these preliminary findings to be translated into practical applications, they need to be supported by advanced studies such as bioavailability, safety, toxicological profiling, and in vivo biological efficacy assessments. With such complementary investigations, *H. montanum* may become a scientifically grounded potential alternative to currently used *Hypericum* species in pharmaceutical and nutraceutical applications.

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