

## Comparative Evaluation of Phenolic Compounds of Flowers, Leaves, and Stems of Wild *Hypericum perforatum* L. in Türkiye

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**Abstract:** The phytochemical content of medicinal and aromatic plants is important for evaluating their biological activity and pharmacological efficacy. In this study, *Hypericum perforatum* L. (St. John's wort), a plant that grows naturally in many regions of Türkiye, was collected from the Çankırı (Eldivan) region, and the phenolic compounds of methanol and ethyl acetate extracts from the flower, leaf, and stem parts were investigated. Phenolic compounds were identified using LC-MS/MS. All analyses were performed in triplicate, and the results were expressed as mean  $\pm$  standard deviation. According to the findings, different parts of the same plant showed significant differences in phenolic compound content depending on the solvent used. In methanol extracts, quinic acid, catechin, epicatechin, and rutin were the compounds with the highest concentrations. The highest concentrations of quinic acid (12977.78  $\mu\text{g/g}$ ), catechin (5675.56  $\mu\text{g/g}$ ), epicatechin (18400  $\mu\text{g/g}$ ), and rutin (9244.44  $\mu\text{g/g}$ ) were determined in the methanol stem extract. In the study, the compound content identified in the methanol extract was higher than that in the ethyl acetate extract. However, the highest amounts of phenolic compounds were detected in the methanol stem extract among the methanol and ethyl acetate extracts. According to the LC-MS/MS results, methanolic extracts exhibited a higher phenolic compound content.

**Keywords:** *Hypericum perforatum* L., St. John's wort, phenolic compounds, LC-MS/MS, Türkiye © 2025 ACG Publications. All rights reserved.

### 1. Introduction

*Hypericum perforatum* L. (St. John's wort) is one of the most widely studied medicinal plants worldwide due to its bioactive metabolites and pharmacological activities [1]. *Hypericum* is one of the oldest genera in the Hypericaceae family and includes approximately 500 species that have been used in folk medicine for centuries [2]. *H. perforatum* L. (St. John's wort), considered the most important species of this genus, has been reported to be used in the treatment of mild to moderate depression due to its antidepressant effects, as well as in the treatment of skin wounds, eczema, and burns due to its antibacterial, antifungal, antiviral, anti-inflammatory, and antioxidant properties [3, 4, 5].

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## Comparative evaluation of wild *Hypericum perforatum*

The therapeutic properties of medicinal plants are mainly dependent on their phytochemical content [6, 7]. Current literature contains data on the morphological, genetic, and chemical diversity of *Hypericum* species. However, the environmental effects on the chemical composition of these species remain poorly understood. Factors influencing chemical diversity in *Hypericum* species include the plant part used in extraction, the phenological stage of the harvested material, the altitude of the growing area, genetic structure, and various biotic and abiotic stress factors [8]. For example, it has been stated that the phytochemical profiles of *H. perforatum* obtained from different geographical regions, seasonal conditions, and soil characteristics show significant differences [9].

*Hypericum* species, especially *H. perforatum*, have been included in the European Pharmacopoeia since ancient times due to the active compounds they contain [10]. Although field cultivation is used to produce standardized medicines, wild-harvesting practices continue, especially in Türkiye. Raw plant material collected from nature and dried in Türkiye is largely exported to Europe, where it is processed and marketed. This situation underscores the importance of *H. perforatum* individuals naturally occurring in the flora of Türkiye and enhances the value of scientific research on these materials [11]. *H. perforatum* L. is still of interest today, and recent clinical and experimental research has shown that this species may be useful in the treatment of diabetes, cancer, rheumatism, digestive system disorders, hepatitis, bronchitis, dysentery, and throat infections [12]. St. John's wort is consumed in forms such as tea and oil, but studies on the phenolic compound content of *H. perforatum* grown in Türkiye are quite limited [13].

*H. perforatum* contains numerous phytochemicals with multidirectional biological effects [14]. Aerial parts of *H. perforatum* L. comprise a wide range of phenolics such as chlorogenic acid, vanillic acid, rosmarinic acid, gallic acid, p-coumaric acid, protocatechuic acid, sinapic acid, 3,4-dihydroxyphenylacetic acid, 2,5-dihydroxybenzoic acid, hydroxybenzoic acid, caffeic acid, syringic acid, 3-hydroxybenzoic acid, verbascoside, and ferulic acid [15]. Phytochemical research has revealed that the compound profiles of different *Hypericum* species can vary considerably. This variability is significantly influenced by the plant's examined organ, developmental stage, genetic characteristics, as well as biotic and abiotic elements of the growth environment (such as geographical location, light and temperature conditions, radiation, soil moisture and salinity, pathogens, and herbivore pressure) [8]. The aim of this study is to investigate the phenolic composition of extracts obtained from the flowers, leaves, and stems of *H. perforatum* L. naturally growing in the Eldivan district of Çankırı, Türkiye, using different solvents (methanol and ethyl acetate) and analyzed by LC-MS/MS.

## 2. Materials and Methods

### 2.1. Plant Material

The *H. perforatum* L. plant was collected in mid-August 2024 from the plateau in the Eldivan district of Çankırı province, during its flowering period, after obtaining the necessary permits. The collection site was at an altitude of approximately 1530 m, with geographical coordinates 40.4845° N, 33.4860° E (Türkiye). The plant material was identified by Dr. Bilal Şahin from Çankırı Karatekin University, and an identification record number (No: BilalŞahin 8299) was assigned.

### 2.2. Phenolic Compounds

Plant samples were separated into flowers, leaves, and stems, and dried in the shade at room temperature until they reached a constant weight. For extract preparation, 5 g of each sample group was mixed with 100 mL of solvent (methanol and ethyl acetate) (1:20 w/v) and incubated at room temperature for 24 h. The mixtures were filtered through Whatman No. 1 filter paper, and the solvents were removed using a rotary evaporator. The extraction procedure was performed in triplicate. The resulting extracts were stored in dark glass bottles at +4°C until analysis. Phenolic compound profiles were subsequently determined using the LC-MS/MS method.

### 2.3. LCMS/MS Analysis

All reference standards, as well as formic acid (with a purity of  $\geq 95\%$ ), were obtained from Sigma-Aldrich. LC-MS grade methanol was sourced from Isolab, while deionized water was produced using a Millipore Direct-Q® 3 UV purification system. The samples were appropriately diluted in a 1:1 methanol–deionized water mixture, then filtered using a PTFE membrane filter (Isolab, 0.45  $\mu\text{m}$  pore size), and subsequently transferred into sealed vials. The analytical method was designed for the qualitative and quantitative determination of 20 phenolic and flavonoid compounds. The analysis was carried out using a liquid chromatography system (Spark Holland) coupled with a tandem mass spectrometer (AB SCIEX 4000 QTRAP). For chromatographic separation, a C18 column (Inertsil ODS-3V, 250 mm  $\times$  4.6 mm, 5  $\mu\text{m}$  particle size) was used. The mobile phase consisted of 0.1% (v/v) formic acid in water (solvent A) and methanol (solvent B). Injection volume was adjusted to 10  $\mu\text{L}$ , flow rate to 0.700 mL/min, and column oven temperature to 30 °C. The total run time for the chromatographic analysis was 20 minutes. Analytes were detected and quantified using Multiple Reaction Monitoring (MRM) mode. Electrospray ionization (ESI) served as the ionization method. The ESI source parameters were set as follows: curtain gas (CUR) at 10 psi, collision gas (CAD) at medium level, ion spray voltage at -4500 V, source temperature at 100 °C, ion source gas 1 (GS1) at 40 psi, and ion source gas 2 (GS2) at 60 psi. The interface heater (IHE) was activated, and a Turbo Spray ion source was employed during ionization. Phytochemical content analysis was carried out using LC–MS/MS, yielding retention times ranging from 3.84 to 17.25 minutes and multiple peaks corresponding to various phenolic compounds. The laboratory's analytical parameters indicated high linearity, with correlation coefficients ( $R^2$ ) exceeding 0.9989 for all quantified compounds. The LOD and LOQ values ranged from 0.38 to 3.53 ng/mL and 1.15 to 10.69 ng/mL, respectively, and the calibration ranges were established between 39 and 20,000 ng/mL.

### 2.4. Multivariate Statistical Analysis

Phenolic compound data were analyzed in RStudio (v.4.5.0). A heatmap was used to visualize the relationships between extracts and phenolic compounds. The data were normalized using Z-score standardization and hierarchically clustered using Euclidean distance and complete linkage. The visualization was done using the heatmap (v.1.0.12) package, and the color scale shows low intensities in blue and high intensities in red. PCA was performed using factominer, factoextra, and gplot2 packages. The first two components were plotted by considering the explanatory variance ratios of the components.

## 3. Results and Discussion

### 3.1. Phenolic Compounds

The phenolic compounds from the *H. perforatum* L. plant grown in the Çankırı region were analyzed by LC-MS/MS, and the results are presented in Table 1. As a result of LC-MS/MS analysis, 20 phenolic compounds were determined in the plant extract (Table 1). The chromatograms of methanol and ethyl acetate extracts and the standard mixture (20000 ppb) are shown in Figures 1 and 2. The quantitative results for the samples are presented in Table 1.

According to LC-MS/MS results, quinic acid, catechin, epicatechin, rutin, and hesperidin are the compounds with the highest concentrations in methanol extracts. However, the highest concentrations of quinic acid (12977.78  $\mu\text{g/g}$ ), catechin (5675.56  $\mu\text{g/g}$ ), epicatechin (18400  $\mu\text{g/g}$ ), rutin (9244.44  $\mu\text{g/g}$ ) and hesperidin (702.22  $\mu\text{g/g}$ ) were determined in the methanol stem extract (Table 1). Similar increases have been reported in the flower, leaf, and stem quinic acid at 2003.52, 1357.02, and 4954.79  $\mu\text{g/g}$ , respectively, and (+)-catechin at 1193.36, 460.65, and 1628.20  $\mu\text{g/g}$ , respectively [16]. The relatively high values observed in some compounds, such as epicatechin and quinic acid, may be related to the study-specific sampling conditions and the extraction method used. In the methanolic extract of *H. perforatum*, the main flavonoids were isoquercitrin (4162  $\mu\text{g/g}$ ), quercetin (874  $\mu\text{g/g}$ ), and hyperoside (636  $\mu\text{g/g}$ ), while chlorogenic acid (100  $\mu\text{g/g}$ ) and rutin (21.2  $\mu\text{g/g}$ ) were relatively lower. These values were reported to be different from those reported for other European populations [17]. In the full-flowering plant period, *H. perforatum* was found to contain hyperoside (18726.59  $\mu\text{g/g}$ ), isoquercitrin (11895.02  $\mu\text{g/g}$ ), rutin (9573.17  $\mu\text{g/g}$ ), and chlorogenic acid (612.38  $\mu\text{g/g}$ ) [18]. The high phenolic levels

Comparative evaluation of wild *Hypericum perforatum*

observed in stem tissues may be related not only to extraction efficiency but also to tissue-specific biological characteristics.

Ethyl acetate flower and ethyl acetate leaf extracts have similar phenolic profiles. Although chlorogenic acid, catechin, and epicatechin showed some variation among the extracts, their levels in the ethyl acetate extract remained largely below the LOQ. Therefore, these compounds are not definitive indicators. This situation indicates that the solvent may limit the distinctive effects of these compounds and that they can be considered potential contributors to the differentiation of extracts. Compared with previous studies, (+)-catechin has been reported to occur in higher amounts in the stem than in many *Hypericum* species and plant parts [19]. Similarly, in this study, (+)-catechin accumulated at higher levels in the stem than in other parts of the plant, and the results are consistent with the literature. When comparing methanol and ethyl acetate extracts in terms of phenolic content, the highest content was found in methanol stem extracts. The pharmacological potential of *H. perforatum* extracts is determined based on their phytochemical composition and the ratios between these compounds so changes in composition may affect the therapeutic efficacy of the extracts [20, 21]. In this study, the data obtained indicate that the stems contain the highest concentration of the compounds examined. The amount of different bioactive compounds contained in *H. perforatum* may vary depending on environmental and ecological factors related to its habitat [22].

**Table 1.** Secondary metabolite screening results

Compounds	RT	Concentration					
		<sup>a</sup> M <sub>flower</sub>	M <sub>leaf</sub>	M <sub>stem</sub>	<sup>b</sup> E <sub>flower</sub>	E <sub>leaf</sub>	E <sub>stem</sub>
Quinic acid	3.84	4153 ± 96.15	3780 ± 246.8	12978 ± 214	39.6 ± 4.08	29.64 ± 0.51	334.7 ± 24.06
Pyrogallol	8.66	18.04 ± 1.94	8.91 ± 0.86	21.37 ± 2.29	6.6 ± 0.06	7.26 ± 0.52	5.02 ± 0.27
Gallic acid	9.10	46.87 ± 1.12	15.93 ± 1.71	38.87 ± 2.88	4.57 ± 0.31	13.28 ± 0.51	5.18 ± 0.47
Catechin <sup>d</sup>	11.58	5027 ± 38.18	2547 ± 161.8	5676 ± 113	D <sup>c</sup>	D <sup>c</sup>	D <sup>c</sup>
Epicatechin <sup>e</sup>	12.56	2800 ± 17.64	3664 ± 107.7	18400 ± 290	23.62 ± 0.57	13.75 ± 1.61	D <sup>c</sup>
Chlorogenic acid <sup>f</sup>	12.62	2.53 ± 0.43	D <sup>c</sup>	4.91 ± 0.56	-	-	D <sup>c</sup>
Salicylic acid	13.04	20.98 ± 1.35	5.62 ± 0.84	10.67 ± 1.99	21.22 ± 2.52	9.58 ± 0.45	12.07 ± 1.04
4-Hydroxybenzoic acid	13.08	22.16 ± 1.54	4.4 ± 0.13	11.76 ± 1.24	22.08 ± 2.24	7.98 ± 1.27	11.47 ± 1.52
Vanillic acid	13.34	30.02 ± 2.14	9.17 ± 0.73	82 ± 1.76	0.73 ± 0.06	3.76 ± 0.96	99.33 ± 7.67
Syringic acid	13.45	2.6 ± 0.06	3.76 ± 0.54	13.15 ± 2.01	1.01 ± 0.13	1.06 ± 0.41	2.55 ± 0.47
Vanillin	13.97	7.11 ± 1.31	4.6 ± 0.48	8.98 ± 0.62	4.96 ± 0.21	9.93 ± 1.83	170.2 ± 9.46
Sinapinic acid	14.57	0.46 ± 0.14	0.37 ± 0.07	0.86 ± 0.17	0.46 ± 0.03	0.48 ± 0.04	0.43 ± 0.16
<i>p</i> -Coumaric acid	14.64	20.08 ± 1.11	14.33 ± 1.33	25.33 ± 0.73	0.49 ± 0.08	3.84 ± 0.51	13.24 ± 0.39
2,5-Dihydroxybenzoic acid	14.91	0.98 ± 0.08	0.99 ± 0.06	4.06 ± 0.37	0.43 ± 0.03	0.41 ± 0.01	0.47 ± 0.04
Hesperidin	15.18	167.6 ± 4.29	610 ± 8.51	702.2 ± 10.18	1.35 ± 0.12	3.66 ± 0.48	79.11 ± 3.85
Rutin	15.19	2331 ± 36.72	8089 ± 367.17	9244 ± 101.8	22.04 ± 0.47	57.78 ± 4.91	1251 ± 95.29
Rosmarinic acid	15.88	41.67 ± 4.81	21.58 ± 2.21	17.89 ± 1.87	11.38 ± 1.43	11.98 ± 2.11	8.22 ± 0.95
Myricetin	16.04	95.78 ± 0.77	96.22 ± 1.54	99.33 ± 6.93	97.56 ± 1.92	95.78 ± 0.77	98.00 ± 2.91
Quercetin	17.22	74.44 ± 1.39	8.07 ± 0.13	55.24 ± 2.68	3.62 ± 0.95	2.62 ± 0.21	3.15 ± 0.21
Naringenin	17.25	2.33 ± 0.23	1.31 ± 0.11	2 ± 0.11	0.97 ± 0.15	0.91 ± 0.03	1.51 ± 0.03

<sup>a</sup>M: Methanol;

<sup>b</sup>E: Ethyl acetate;

<sup>c</sup>Peak observed, however, concentration was lower than the Limit of Quantification;

<sup>d</sup>LOD/LOQ: 2.88/8.74 nG/mL;

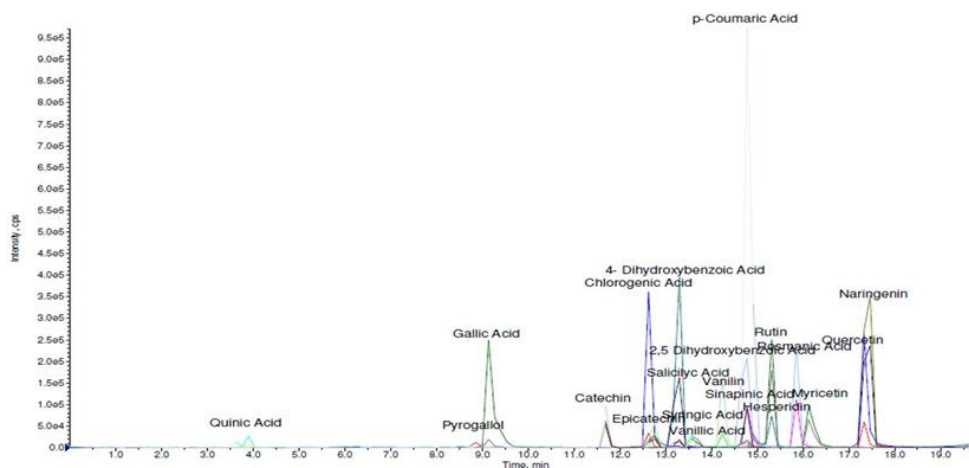
<sup>e</sup>LOD/LOQ: 2.19/6.66 nG/mL;

<sup>f</sup>LOD/LOQ: 0.83/2.52 nG/mL

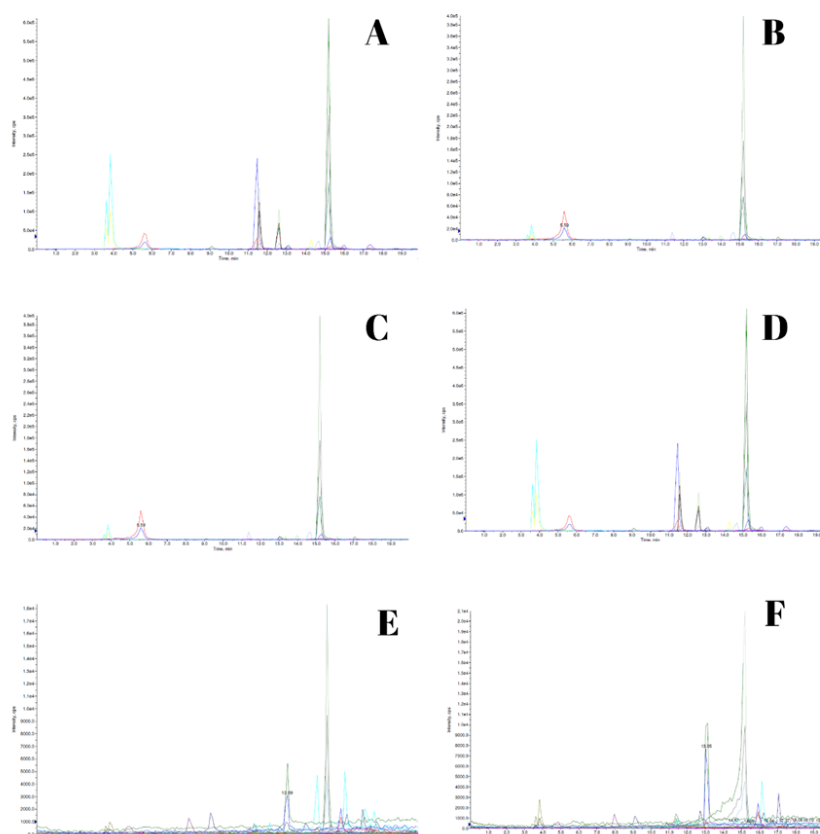
Pharmaceuticals and dietary supplements derived from *H. perforatum* L. currently rank 37th among the best-selling herbal products and are on track to become one of the top 40 best-selling products in the natural/whole food/lifestyle category in the international market [23]. Therefore, the phenolic compounds identified in this study provide baseline chemical information that may be useful for future studies evaluating pharmacological, therapeutic, and biological activities.

### 3.2. PCA and Heatmap Analysis

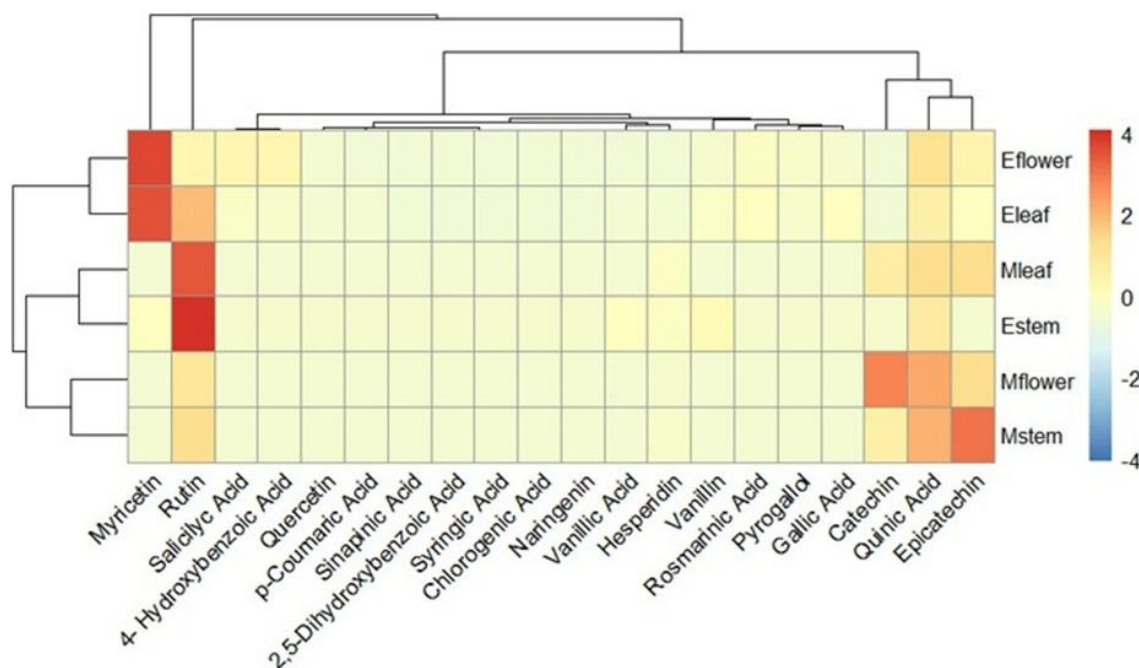
The heatmap and dendrogram analysis presented in Figure 3 clearly reflect the phenolic profiles of different plant parts and extraction solvents. In particular,  $M_{\text{stem}}$  showed a high correlation with quinic acid, catechin, and epicatechin, highlighting the phenolic richness of stem samples. Similarly,  $M_{\text{flower}}$  was also clustered with these compounds. On the other hand,  $E_{\text{flower}}$  and  $E_{\text{leaf}}$  samples were clustered with myricetin and rutin. This indicates that the ethyl acetate solvent provides a different phenolic compound profile.



**Figure 1.** Standard chromatogram (20000 ppb)



**Figure 2.** LC-MS/MS chromatograms of A: Methanol<sub>flower</sub>, B: Methanol<sub>stem</sub>, C: Methanol<sub>leaf</sub>, D: Ethyl acetate<sub>flower</sub>, E: Ethyl acetate<sub>stem</sub>, F: Ethyl acetate<sub>leaf</sub>

Comparative evaluation of wild *Hypericum perforatum*

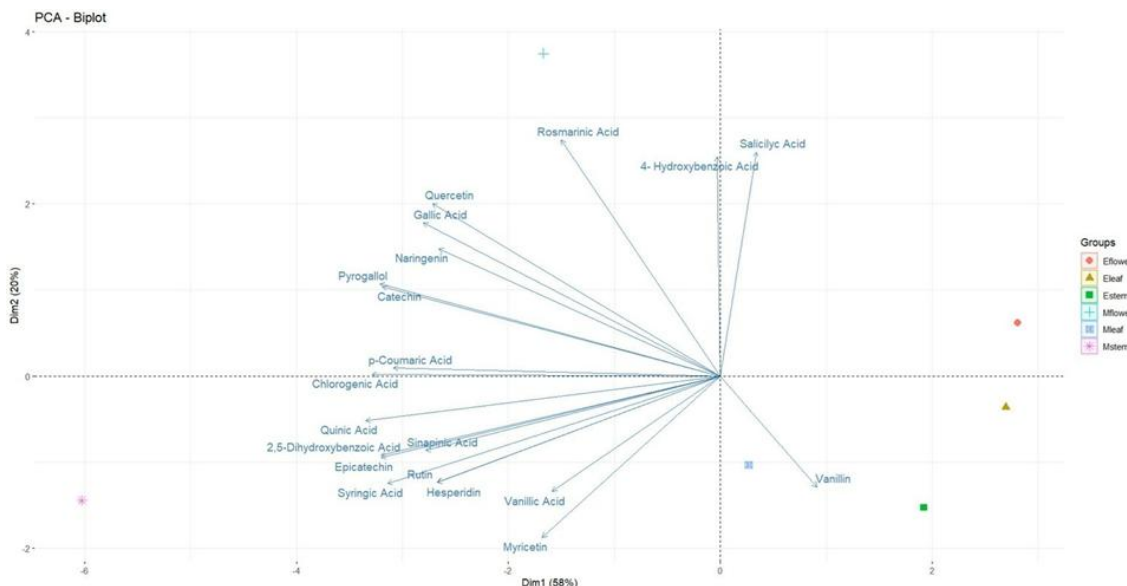
**Figure 3.** Heatmap based on the phenolic compound content of flower, leaves, and stems extracts from methanol and ethyl acetate extracts

**Table 2.** Loading value, variance, and cumulative values for the relationship between phenolic compounds

Variable	PC1	PC2
Quinic acid	-0,287	-0,075
Pyrogallol	-0,276	<b>0,156</b>
Gallic acid	-0,240	<b>0,260</b>
Catechin	-0,274	<b>0,151</b>
Epicatechin	-0,275	-0,138
Chlorogenic acid	-0,282	<b>0,002</b>
Salicylic acid	<b>0,029</b>	<b>0,380</b>
4-Hydroxybenzoic acid	-0,002	<b>0,372</b>
Vanillic acid	-0,136	-0,196
Syringic acid	-0,270	-0,182
Vanillin	<b>0,078</b>	-0,188
Sinapinic acid	-0,239	-0,126
p-Coumaric acid	-0,265	<b>0,013</b>
2,5-Dihydroxybenzoic acid	-0,275	-0,134
Hesperidin	-0,229	-0,180
Rutin	-0,229	-0,180
Rosmarinic acid	-0,129	<b>0,401</b>
Myricetin	-0,144	-0,274
Quercetin	-0,233	<b>0,293</b>
Naringenin	-0,228	<b>0,216</b>
Value	11,6032	4,0090
Variance (%)	58,016	20,045
Cumulative (%)	58,016	78,061

Principal component analysis (PCA) was applied to visualize the distribution and relationships of phenolic compounds among samples in two dimensions. Biplot plots generated from LC-MS/MS data

revealed correlations between samples and phenolic compounds. According to the PCA results, Dim 1 (58%) and Dim 2 (20%) explained 78% of the total variance, indicating a clear divergence between sample groups (Figure 4 and Table 2). The extracts were distributed across different regions depending on the plant part and the extraction solvent used. Methanol stem extracts were separated from other groups, particularly due to the influence of major compounds such as quinic acid, catechin, and epicatechin. Flower extracts were in the same region as rosmarinic acid, salicylic acid, and 4-hydroxybenzoic acid. Vanillin showed a more significant contribution in the ethyl acetate stem extract. In contrast, leaf samples exhibited a more balanced distribution of phenolic compounds.



**Figure 4.** PCA analysis of phenolic compounds in methanol and ethyl acetate extracts of *H. perforatum* flowers, leaves, and stems

#### 4. Conclusion

The phenolic compound profiles of methanol and ethyl acetate extracts obtained from the flower, leaf, and stem parts of *H. perforatum* were characterized and compared. The findings revealed that these extracts are rich in phenolic compounds, including rutin, quinic acid, epicatechin, and catechin. The highest phenolic compound content for both solvents was observed in the stem extracts, while the highest total phenolic content was detected in the methanol extracts. In line with these results, the study provides basic data on the chemical profile of these compounds and contributes to the understanding of regional phytochemical diversity. However, this study was conducted in a specific location and during a specific vegetative period, and the results were interpreted in the context of the conditions examined. Future studies are expected to contribute to a broader analysis of these findings under different samples and experimental conditions.

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#### Conflict of Interest

Conflict of Interest The authors declare that they have no competing financial interests or personal relationships that could have influenced the work reported in this paper.



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