

RESEARCH ARTICLE

Phenolic Composition, Chemometric Analysis, and Multifunctional Biological Activities of Organ-Specific Extracts From Endemic *Hedysarum erythroleucum*

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ABSTRACT

Hedysarum erythroleucum is a plant species native to Türkiye and represents a potential source of bioactive compounds. This study investigated the phytochemical composition and biological activities of methanolic extracts prepared from the leaves, stems, and roots. LC-MS/MS analysis identified 53 phenolic compounds and revealed clear differences in metabolite distribution among plant organs. Quinic acid was the predominant phenolic acid, particularly in the leaf extract. Flavonoid glycosides such as isoquercitrin, astragalin, and rutin were also abundant in the aerial parts of the plant. The antioxidant potential of the extracts was evaluated using DPPH, FRAP, total phenolic content, and total flavonoid content assays. Among the tested plant parts, the leaf extract exhibited the strongest antioxidant activity, with the lowest DPPH IC₅₀ value, the highest reducing power, and the highest total phenolic and flavonoid contents. These findings indicate that the leaves contain high levels of antioxidant compounds. Enzyme inhibition assays showed that the leaf extract exhibited the highest inhibitory activity against acetylcholinesterase, butyrylcholinesterase, and α -glycosidase. Its activity was comparable to that of the reference inhibitors under the experimental conditions. Antiproliferative activity was assessed against lung and breast cancer cell lines. All extracts inhibited cancer cell growth, with the highest sensitivity observed in H1650 and MDA-MB-231 cells. Tumor selectivity indices suggested moderate selectivity, especially for root extracts. LDH cytotoxicity analysis indicated limited membrane damage, suggesting that the extracts primarily exert cytostatic effects by suppressing cell proliferation rather than inducing extensive cell death. Multivariate analyses revealed organ-specific metabolite patterns and associations between phenolic composition and the measured biological activities. Overall, the findings indicate that *H. erythroleucum*, particularly the leaf extract, contains a high content of phenolic compounds and exhibits promising in vitro antioxidant, enzyme inhibitory, and antiproliferative activities. These findings provide a basis for future phytochemical investigations, mechanistic studies, compound isolation, and in vivo validation.

1 | Introduction

The use of medicinal plants as therapeutic agents has a long history, and plant-derived bioactive compounds continue to attract considerable attention in pharmaceutical and nutraceutical research. This increasing interest is supported by numerous studies demonstrating that secondary metabolites, including phenolic acids, flavonoids, alkaloids, and terpenoids, exhibit various pharmacological properties such as antioxidant, anti-inflammatory, anticancer, antidiabetic, and neuroprotective activities (Dong et al. 2013; Tohma et al. 2016). Increasing concerns about the safety and high costs of synthetic pharmaceuticals have further stimulated interest in phytochemically important plant species as natural alternatives or complementary approaches for disease management (Zengin et al. 2015; Altay et al. 2022). In regions with high botanical diversity, such as Anatolia, which includes over 11,000 plant species, approximately one-third of which are endemic, the native flora represents an important resource for drug discovery (Bektaş et al. 2018). The integration of liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) and multiparameter bioactivity screening supports the association of organ-specific metabolite profiles with pharmacological effects (Altay et al. 2022; Keleke et al. 2025).

Neurodegenerative disorders, metabolic diseases, and cancer are among the major challenges in global public health due to their increasing prevalence (Taslimi and Gulçin 2017; Durmaz et al. 2022; Topal and Gulcin 2022). Alzheimer's disease (AD), the most common cause of dementia in more than 55 million individuals, is associated with the cholinergic hypothesis, in which progressive degradation of acetylcholine by acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) results in cognitive impairment (Melrose 2023; ALNasser et al. 2025). Currently approved synthetic cholinesterase inhibitors provide only symptomatic treatment and are associated with important safety concerns, indicating the need for safer plant-derived alternatives (Kaur et al. 2022). Type 2 diabetes mellitus (T2DM) represents a major health problem worldwide, with over 537 million reported cases. Inhibition of α -glucosidase is a widely accepted approach for reducing postprandial blood glucose levels; however, first-line inhibitors such as acarbose are associated with gastrointestinal complications that may reduce patient compliance (Kashtoh and Baek 2022; Aryal et al. 2024). In addition, according to GLOBOCAN 2022 estimates, about 20 million new cancer cases and 9.7 million cancer-related deaths were recorded worldwide, with lung cancer accounting for 12.4% of all new cases and representing the main cause of cancer-related mortality, while breast cancer was the second most commonly diagnosed malignancy (Bray et al. 2024). Plant-derived secondary metabolites represent important antiproliferative agents because of their ability to modulate multiple oncogenic pathways and their lower toxicity compared with conventional cytotoxic agents.

The family Fabaceae includes numerous plant species with biological activities that have contributed to both traditional medicine and modern drug development. Within this family, the genus *Hedysarum* L. (sweetvetch) includes about 154–300 species distributed in Europe, Asia, North Africa, and North America and is recognized for its ecological and ethnopharmacological importance (Dong et al. 2013; Keleke et al. 2025).

In traditional Chinese medicine, several *Hedysarum* species have been associated with immunomodulatory and tissue-regenerative applications, while Mediterranean representatives of the genus have been used in traditional practices for astringent, laxative, and antihypercholesterolemic purposes (Tava et al. 2021; Altay et al. 2022). In Türkiye, the genus is represented by 21 species that show the phytogeographic characteristics of the Anatolian flora, making Turkish *Hedysarum* species important subjects for systematic phytochemical investigation (Bektaş et al. 2018; Altay et al. 2022).

Comprehensive phytochemical investigations of *Hedysarum* have identified more than 155 compounds, including flavonoids, phenolic acids, triterpenes, coumarins, lignans, and polysaccharides, with flavonoids recognized as one of the major pharmacologically important metabolite classes (Dong et al. 2013; Altay et al. 2022). Previous studies on Turkish *Hedysarum* species have reported various phytochemical profiles and biological activity evaluations, particularly involving flavonoids, quinic acid derivatives, kaempferol derivatives, and other phenolic compounds identified in different plant organs (Altay et al. 2022; Dob et al. 2024; Baltürk et al. 2025; Keleke et al. 2025). Although research on *Hedysarum* has increased, many Turkish species, particularly endemic species from Eastern Anatolia, have not yet been investigated phytochemically, and information on their chemical diversity and biological activities is still limited.

The pharmacological importance of phenolic compounds and flavonoids is recognized across all major disease categories. Quercetin and its glycosides have been reported as potent inhibitors of both AChE and BChE and have shown neuroprotective effects through antioxidant, anti-neuroinflammatory, and antiamyloidogenic mechanisms relevant to AD pathogenesis (Zaplatic et al. 2019; Lee et al. 2023). Kaempferol derivatives and miquelianin (quercetin-3-O-glucuronide) have demonstrated inhibitory potential against both cholinesterase and carbohydrate-hydrolyzing enzymes (Kashchenko et al. 2017; Li et al. 2023). Isoquercitrin, rutin, and hesperidin—major constituents identified in several *Hedysarum* species—have been reported to inhibit α -glucosidase activity and reduce neuronal oxidative damage, supporting their potential relevance to both T2DM and AD (Yang et al. 2021; Melrose 2023). Quinic acid, consistently present at high concentrations in the aerial parts of *Hedysarum* species, has shown neuroprotective effects by modulating the p38 MAPK pathway and antidiabetic potential by regulating glucose metabolism (Benali et al. 2024; Rafi et al. 2025). In anticancer research, quercetin, kaempferol, and their glycosides have been associated with apoptosis induction and inhibition of oncogenic signaling pathways, including PI3K/AKT and MAPK, in different malignant cell lines (Dong et al. 2013; Lee et al. 2023), and phytochemically complex extracts containing high flavonoid content have therefore been considered potential sources for antiproliferative drug discovery studies.

HE is a species endemic to Türkiye, naturally distributed in arid and semiarid regions of Eastern and Central Anatolia at high elevations. Although this species is distributed in one of the botanically important regions of the Western Palearctic, no studies on its phytochemical composition, biological activities, or organ-specific metabolite profile have been reported in the

scientific literature to date. This represents an important gap, particularly considering the pharmacological potential reported for related Turkish *Hedysarum* species. Endemic plants adapted to ecologically challenging environments often contain high concentrations of protective secondary metabolites as constitutive responses to abiotic stressors, increasing their value as sources of pharmacologically important compounds (Isah 2019; Yeshe et al. 2022). The limited comprehensive multiorgan phytochemical profiling of many Turkish *Hedysarum* species indicates that the chemophenetic characterization of this genus in Türkiye is still incomplete, limiting both taxonomically informed drug discovery and the rational selection of candidate species for preclinical development.

To the best of our knowledge, the present study provides the first comprehensive biological and chemical characterization of HE collected from the Erzincan region of Türkiye. Methanolic extracts obtained from root, stem, and leaf samples were analyzed by quantitative LC-MS/MS for 53 phenolic and flavonoid compounds, and organ-specific metabolite distribution was determined for this species. The biological activity panel included (i) antioxidant capacity determined using DPPH and ferric reducing antioxidant power (FRAP) assays together with total phenolic and flavonoid quantification; (ii) enzyme inhibitory activity against AChE, BChE, and α -glycosidase using tacrine and acarbose as reference compounds; and (iii) antiproliferative and cytotoxic effects tested in lung (A549, Calu-1, and H1650) and breast (MCF7, MDA-MB-231) cancer cell lines with corresponding noncancerous controls (Beas2B and MCF10A) for tumor selectivity index (TSI) calculations, together with LDH cytotoxicity assay results. The integration of LC-MS/MS metabolite profiling with these bioactivity analyses provides a reference for the pharmacological evaluation of HE and contributes to current phytochemical knowledge of *Hedysarum* in Türkiye, with potential relevance for pharmaceutical and nutraceutical applications.

2 | Materials and Methods

2.1 | Plant Material

The plant material of HE was collected in 2024 from its natural habitat in the Ergen locality of Erzincan (Türkiye), during the flowering period. The collected specimens were separated into root, stem, and leaf parts and dried in the shade under laboratory conditions. The plant was taxonomically identified by Prof. Dr. Ali Kandemir (Department of Biology, Faculty of Arts and Sciences, Erzincan Binali Yıldırım University). A voucher specimen has been deposited in the EBYU Herbarium (Herbarium No.: 0000047).

2.2 | Extraction Procedure

The dried plant materials (root, stem, and leaf) were ground into a fine powder, and 10 g of each sample was extracted with 100 mL of methanol by overnight maceration at room temperature. Due to its polarity and suitability for extracting phenolic and flavonoid compounds, methanol was selected as the extraction

solvent (Kiliç et al. 2025). The extraction, repeated three times, resulted in extracts that were combined. The mixtures, filtered to remove plant residues, were subjected to solvent evaporation under reduced pressure to obtain crude extracts. The extracts were stored in dark vials at 4 °C until further analysis.

2.3 | LC-MS/MS Conditions

The quantitative profiling of phytochemicals in the methanolic extracts obtained from HE leaves, stems, and roots was performed using LC-MS/MS according to a previously validated analytical procedure (Akman, Şimşek, Akşit, Akşit, et al. 2024; Aslan et al. 2025; Yapıcı et al. 2025; Yılmaz et al. 2025) on a Shimadzu Nexera ultrahigh-performance liquid chromatography (UHPLC) system coupled with a tandem mass spectrometer. The analytical method had been validated with respect to linearity, limits of detection (LOD), limits of quantification (LOQ), recovery, and precision under the same instrumental conditions, as described in the referenced studies. Equipped with an autosampler (SIL-30AC), a column oven (CTO-10ASvp), binary pumps (LC-30AD), and a degasser (DGU-20A3R), a reversed-phase UHPLC system was used. Chromatographic separation was performed on a reversed-phase Agilent Poroshell 120 EC-C18 analytical column (150 × 2.1 mm, 2.7 µm) at 40 °C. Elution was conducted using a binary mobile phase system consisting of water containing 5 mM ammonium formate and 0.1% formic acid as solvent A and methanol containing 5 mM ammonium formate and 0.1% formic acid as solvent B. The gradient elution program was as follows: 20%–100% B from 0 to 25 min, 100% B from 25 to 35 min, and 20% B from 35 to 45 min. The flow rate was set at 0.5 mL/min, and the injection volume was 5 µL.

Mass spectrometric analysis was conducted using a Shimadzu LCMS-8040 tandem mass spectrometer equipped with an electrospray ionization (ESI) source operating in both positive and negative ionization modes (Akman, Şimşek, Akşit, Aydın, et al. 2024). Data acquisition and processing were performed using LabSolutions software (Shimadzu). For the selective identification and quantification of phytochemicals, the multiple reaction monitoring (MRM) mode was used based on optimized precursor-to-product ion transitions. Collision energies (CEs) were optimized for each analyte to increase the intensity of the characteristic product ions and improve ion transmission. The MS operating conditions were as follows: drying gas (N₂) flow rate of 15 L/min, nebulizing gas (N₂) flow rate of 3 L/min, desolvation line (DL) temperature of 250 °C, heat block temperature of 400 °C, and interface temperature of 350 °C.

2.4 | Enzyme Assays

2.4.1 | Anticholinesterase Activity

The inhibitory activities of the plant extracts against AChE and BChE were determined spectrophotometrically according to a previously reported protocol, with minor modifications (Tüfekçi et al. 2023; Akman, Şimşek, Erken, et al. 2025; Köksal et al. 2025). Stock solutions of the plant extracts and tacrine (used as a positive control) were prepared in deionized water

and diluted to obtain a series of concentrations. The reaction mixture consisted of 100 μ L Tris–HCl buffer (1 M, pH 8.0), 50 μ L 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB), and 20 μ L enzyme solution. The enzymatic reaction was initiated by adding acetylthiocholine iodide (for AChE) or butyrylthiocholine iodide (for BChE) as the substrate. Following enzymatic hydrolysis, thiocholine reacted with DTNB to form the yellow-colored 5-thio-2-nitrobenzoate anion. The absorbance of the chromogenic product was recorded at 412 nm. Enzyme inhibitory activity was expressed as IC₅₀ values, representing the concentration of the inhibitor that reduces enzyme activity by 50%. All assays were performed in triplicate, and the results are presented as mean $\pm$ standard deviation (SD).

2.4.2 | α -Glycosidase Inhibition Activity

α -Glycosidase inhibitory activity was determined using a previously described procedure with minor modifications (Akman et al. 2023; Durmaz et al. 2023; Durmaz et al. 2024). Acarbose (positive control) and plant extracts were diluted with deionized water to concentrations. Phosphate buffer (1 M, pH 7.4), sample, and enzyme solution were mixed and incubated at 35 °C for 10 min. After incubation, *p*-nitrophenyl- α -D-glucopyranoside (*p*-NPG) was added, and at 35 °C, the mixture was incubated again. The absorbance of the reaction product was recorded spectrophotometrically at 405 nm. The inhibitory concentration corresponding to 50% enzyme inhibition was obtained from the concentration–response curves. All experiments were performed in triplicate, and the results are presented as mean $\pm$ SD.

2.5 | Antioxidant Activity

2.5.1 | DPPH Free Radical Scavenging Activity

The DPPH[•] radical scavenging activity of the extracts was determined according to a previously described method (Gulcin and Alwasel 2023; Albayrak et al. 2024; Akşit and Şimşek 2025; Şimşek 2026). Briefly, 3 mL of the test sample at different concentrations was mixed with 1 mL of 0.26 mM DPPH[•] solution. Following vortexing, the reaction mixtures were incubated in the dark for 30 min at room temperature. The absorbance was recorded at 517 nm. The radical scavenging activity was calculated using the following equation: RSA (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 corresponds to the absorbance of the control, and A_1 corresponds to the absorbance in the presence of the extracts or standards. The absorbance values were converted to percentage activity, and the IC₅₀ values (μ g/mL) of the extracts were determined accordingly. The results were evaluated in comparison with standard antioxidants, including Trolox, butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), and ascorbic acid. All analyses were performed in triplicate, and the results are presented as mean $\pm$ SD.

2.5.2 | FRAP

The reducing power activity of HE root, stem, and leaf extracts was investigated according to a previously described method (Alkan et al. 2025; Şimşek 2025b; Şimşek et al. 2026). After preparation of the extract stock solutions (1 mg/mL), 250 μ L

of each sample was combined with phosphate buffer (0.2 M, pH 6.6) to a final volume of 1.25 mL. Following the addition of 1.25 mL of 1% K₃Fe(CN)₆, the mixture was incubated at 50 °C for 20 min. At the end of the incubation period, 1.25 mL of 10% TCA and 0.25 mL of 0.1% FeCl₃ were added to the reaction mixture. The absorbance of the final mixture was recorded at 700 nm, and the results were expressed as mg TE/g extract based on the calibration curve prepared with Trolox (10–100 μ g/mL). The results were evaluated against standard antioxidants, including Trolox, BHT, BHA, and ascorbic acid. All assays were performed in triplicate, and the results are presented as mean $\pm$ SD.

2.5.3 | Total Phenolic Content

The total phenolic content of the extracts was determined using the Folin–Ciocalteu method according to a previously reported procedure (Akşit et al. 2022; Gözcü et al. 2024b; Şimşek 2025a). Briefly, 100 μ L of extract and standard solutions (1 mg/mL) were mixed with 4.5 mL of distilled water. After the addition of 100 μ L Folin–Ciocalteu reagent, 300 μ L of 2% Na₂CO₃ was added to the mixtures. The mixtures were vortexed and incubated at room temperature for 120 min, after which the absorbance was recorded spectrophotometrically at 760 nm. A calibration curve ($y = 0.117x - 0.011$) was prepared using different concentrations of gallic acid (1–1000 μ g/mL). Results for total phenolic content were calculated as mg gallic acid equivalents (GAE) per g extract, and all analyses were performed in triplicate.

2.5.4 | Total Flavonoid Content

According to previously reported methods (Varol et al. 2023; Adigüzelli et al. 2024; Şimşek et al. 2025), total flavonoid content was analyzed spectrophotometrically. First, 100 μ L of extract and standard solutions (1 mg/mL) was added to 4.7 mL of methanol. Following this, 100 μ L NH₄CH₃COO (1 M) solution and 100 μ L AlCl₃ (10%) were added to the reaction mixture, followed by vortexing and incubation at room temperature for 45 min. The absorbance was recorded at 415 nm. Quantification was performed using a calibration curve prepared with quercetin (1–1000 μ g/mL), and the results were expressed as mg quercetin equivalents (QE)/g extract. All experiments were performed in triplicate.

2.6 | Antiproliferative Activity

2.6.1 | Cell Lines and Cell Culture

In this study, human lung cancer cell lines A549 (ATCC CCL-185), Calu-1 (ATCC HTB-54), and H1650 (ATCC CRL-5883) and breast cancer cell lines MCF7 (ATCC HTB-22) and MDA-MB-231 (ATCC HTB-26), together with the normal lung cell line Beas-2B (RRID: CVCL-0168) and the normal breast cell line MCF10A (ATCC CRL-10317), were used. In a laminar flow cabinet, all cell culture procedures were conducted. Cells were cultured in DMEM or RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 2% penicillin–streptomycin and incubated at 37 °C in an atmosphere containing 5% CO₂. Cells (1×10^4 cells/well) were added to 96-well plates. After

16-h incubation, the samples were added, and the cells were incubated for a further 24 h before analysis (Köksal et al. 2021; Akman, Simsek, Aksit, et al. 2025).

2.6.2 | Measurement of Cell Proliferation

The effects of the plant extracts on the proliferation of cancer cell lines after 24 h treatment were determined using the MTT assay (Akşit et al. 2025; Gözcü et al. 2025). The results were expressed as percentage inhibition relative to solvent-treated (DMSO) control cells, which were regarded as 100%. The percentage inhibition was calculated using the following formula: $\% \text{ inhibition} = [1 - (A_{\text{test}}/A_{\text{control}})] \times 100$. IC_{50} values were determined from the absorbance data obtained following treatment with increasing concentrations of the extracts (1.96–125.0 $\mu\text{g/mL}$). Dose–response curves were obtained by logarithmic regression analysis using Microsoft Excel. The GI_{50} values were calculated according to the following equations: $[(T_i - T_z)/(C - T_z)] \times 100$ for $T_i \geq T_z$ (cytostatic effect) and $[(T_i - T_z)/T_z] \times 100$ for $T_i < T_z$ (cytotoxic effect), where T_z represents the absorbance at time zero, C corresponds to the control growth, and T_i denotes the growth in the presence of the test samples. The concentration required to produce 50% growth inhibition was expressed as GI_{50} . The TSI was calculated using the IC_{50} value obtained for normal cells relative to that of cancer cells.

2.6.3 | Cytotoxicity Test

The cytotoxic and cytostatic effects of the extracts were determined using the lactate dehydrogenase (LDH) release assay (Şimşek et al. 2023; Gözcü et al. 2024a). LDH, a stable cytoplasmic enzyme, is released into the culture supernatant following plasma membrane damage; therefore, increased LDH levels indicate increased cell death. The LDH cytotoxicity assay was conducted according to the manufacturer's instructions. Formazan formation resulting from LDH enzymatic activity was quantified spectrophotometrically, and percentage cytotoxicity was calculated using the following equation:

$$\% \text{ Cytotoxicity} = [(A_{\text{sample}} - A_{\text{low control}})/(A_{\text{high control}} - A_{\text{low control}})] \times 100$$

2.6.4 | Statistical Analyses

Statistical analyses were performed using SPSS version 20.0 software (IBM Corp., Armonk, NY, United States). All LC-MS/MS analyses and biochemical assays were performed in triplicate, and the results are presented as mean $\pm$ SD. Significant differences among extracts obtained from different plant organs were determined by one-way analysis of variance (ANOVA), followed by Duncan's multiple range test for post hoc comparisons at $p < 0.05$. To evaluate the distribution patterns of phytochemical constituents and their relationships with the investigated biological activities, multivariate analyses, including principal component analysis (PCA) and hierarchical heatmap analysis, were performed.

3 | Results and Discussion

3.1 | Phytochemical Investigation of the Extracts by LC-MS/MS

Phytochemical profiling of the HE extracts was performed using a previously developed and validated LC-MS/MS method. The extracts were analyzed for their bioactive phytochemical composition, and 53 phenolic compounds were quantified. The quantitative data are presented in Table 1, while Figure 1 shows the LC-MS/MS TIC chromatograms of the HE extracts.

The LC-MS/MS quantification demonstrated that phenolic acids and flavonoid glycosides were the major metabolite groups detected in HE. Among the quantified analytes, quinic acid was present at the highest concentration, particularly in the aerial parts, with the highest concentration observed in the leaves (108.276 mg/g extract) and stems (90.074 mg/g extract) than in roots (9.91 mg/g extract). The high quinic acid concentration in the aerial parts is consistent with previous findings reported for *Hedysarum candidissimum* (Altay et al. 2022), suggesting that this distribution pattern may be conserved within the genus. The higher quinic acid content in leaves may be associated with increased shikimate pathway activity in photosynthetically active plant organs. Considering the diverse biological activities previously reported for quinic acid, its relatively high concentration may have contributed to the biological activities observed in the leaf extract. However, this relationship should be interpreted with caution because the biological activities of individual compounds were not investigated in the present study.

Flavonoid glycosides constituted the major group of secondary metabolites in the aerial organs (Gulcin 2025; Gülçin 2025). Isoquercitrin exhibited the highest concentration among the flavonoids, with concentrations of 35.243 mg/g extract in leaves, 17.691 mg/g extract in stems, and 0.86 mg/g extract in roots. Rutin followed a similar distribution pattern, with concentrations of 6.866 mg/g (leaf), 2.568 mg/g (stem), and 0.613 mg/g (root). Miquelianin was present in leaves (8.303 mg/g extract) and stems (2.12 mg/g extract) but was not found in roots. These quercetin glycosides have been reported to exhibit antioxidant, anti-inflammatory, antihypertensive, antiviral, and cytoprotective activities (Altay et al. 2022). A similar distribution has also been reported for *Hedysarum semenowii*, in which quercetin glycosides were predominantly detected in the aerial organs (Keleke et al. 2025). This distribution pattern is consistent with the reported roles of flavonol glycosides in photoprotection and antimicrobial defense in aerial parts. This distribution pattern is consistent with the reported roles of flavonol glycosides in photoprotection and antimicrobial defense in aerial parts. Accordingly, the higher concentrations of these compounds in the aerial organs may be associated with the stronger biological activities observed for the leaf extract.

Hesperidin also showed organ-dependent variation, with concentrations of 3.674 mg/g extract in leaves, 1.598 mg/g extract in stems, and 0.277 mg/g extract in roots. Astragalin was quantified in leaves (7.641 mg/g extract), stems (2.702 mg/g extract), and roots (0.264 mg/g extract). Nicotiflorin showed concentrations

TABLE 1 | Quantitative LC-MS/MS analysis of phenolic compounds in *Hedysarum erythroleucum* leaf, stem, and root extracts (mg analyte/g extract).

No.	Compound name	RT, min	Leaf	Stem	Root	No.	Compound name	RT, min	Leaf	Stem	Root
1	Quinic acid	3.0	108.276	90.074	9.910	29	Salicylic acid	21.8	0.231	0.564	0.453
2	Fumaric acid	3.9	0.340	0.677	0.481	30	Cyanoside	23.7	N.D.	0.36	N.D.
3	Aconitic acid	4.0	N.D.	N.D.	N.D.	31	Miquelianin	24.1	8.303	2.12	N.D.
4	Gallic acid	4.4	0.238	0.217	0.146	32	Rutin-D3-IS	25.5	N.A.	N.A.	N.A.
5	Epigallocatechin	6.7	N.D.	N.D.	N.D.	33	Rutin	25.6	6.866	2.568	0.613
6	Protocatechuic acid	6.8	0.409	0.391	0.255	34	Isoquercitrin	25.6	35.243	17.691	0.860
7	Catechin	7.4	N.D.	N.D.	N.D.	35	Hesperidin	25.8	3.674	1.598	0.277
8	Gentisic acid	8.3	0.095	0.102	N.D.	36	<i>o</i> -Coumaric acid	26.1	N.D.	N.D.	N.D.
9	Chlorogenic acid	8.4	0.030	0.122	0.057	37	Genistin	26.3	0.098	0.081	0.642
10	Protocatechuic aldehyde	8.5	N.D.	N.D.	N.D.	38	Rosmarinic acid	26.6	N.D.	N.D.	N.D.
11	Tannic acid	9.2	0.526	0.142	0.062	39	Ellagic acid	27.6	N.D.	N.D.	N.D.
12	Epigallocatechin gallate	9.4	N.D.	N.D.	N.D.	40	Cosmosiin	28.2	N.D.	N.D.	N.D.
13	Cynarin	9.8	N.D.	N.D.	N.D.	41	Quercitrin	29.8	3.660	3.025	1.545
14	4-OH benzoic acid	10.5	N.D.	N.D.	N.D.	42	Astragalin	30.4	7.641	2.702	0.264
15	Epicatechin	11.6	N.D.	N.D.	N.D.	43	Nicotiflorin	30.6	3.386	1.629	0.526
16	Vanillic acid	11.8	N.D.	N.D.	N.D.	44	Fisetin	30.6	N.D.	N.D.	N.D.
17	Caffeic acid	12.1	0.022	0.039	0.875	45	Daidzein	34.0	0.019	0.034	0.199
18	Syringic acid	12.6	N.D.	N.D.	N.D.	46	Quercetin-D3-IS	35.6	N.A.	N.A.	N.A.
19	Vanillin	13.9	N.D.	N.D.	N.D.	47	Quercetin	35.7	0.2	0.091	0.070
20	Syringic aldehyde	14.6	N.D.	N.D.	N.D.	48	Naringenin	35.9	0.045	0.05	0.096
21	Daidzin	15.2	0.009	N.D.	0.024	49	Hesperetin	36.7	0.027	0.025	0.014
22	Epicatechin gallate	15.5	N.D.	N.D.	N.D.	50	Luteolin	36.7	0.005	0.008	0.025
23	Piceid	17.2	N.D.	N.D.	N.D.	51	Genistein	36.9	1.207	1.032	1.201
24	<i>p</i> -Coumaric acid	17.8	0.031	0.02	0.003	52	Kaempferol	37.9	0.027	0.012	0.008
25	Ferulic acid-D3-IS ^h	18.8	N.A.	N.A.	N.A.	53	Apigenin	38.2	0.010	0.015	0.114
26	Ferulic acid	18.8	N.D.	N.D.	N.D.	54	Amentoflavone	39.7	N.D.	N.D.	N.D.
27	Sinapic acid	18.9	N.D.	N.D.	N.D.	55	Chrysin	40.5	N.D.	N.D.	N.D.
28	Coumarin	20.9	N.D.	N.D.	N.D.	56	Acacetin	40.7	0.851	0.629	1.329

Abbreviations: IS, internal standard; N.A., not applicable; N.D., not detected.

of 3.386 mg/g extract in leaves, 1.629 mg/g extract in stems, and 0.526 mg/g extract in roots. These kaempferol and quercetin glycosides are associated with antioxidant, anti-inflammatory, cardioprotective, and neuroprotective properties (Altay et al. 2022). The higher concentrations of these flavonoid glycosides in the aerial organs are in agreement with previous reports for *Hedysarum* species (Keleke et al. 2025). The higher concentrations of astragalin and nicotiflorin in the aerial parts may be associated with their contribution to radical scavenging and antimicrobial activities.

Interestingly, isoflavonoid aglycones demonstrated a contrasting distribution. Genistein showed similar concentrations in roots (1.201 mg/g extract), stems (1.032 mg/g extract), and leaves (1.207 mg/g extract), indicating a relatively even distribution among the analyzed plant parts. In contrast, daidzein showed the highest concentration in roots (0.199 mg/g extract) and lower

concentrations in the aerial parts. Isoflavones are widely recognized for their estrogenic, antioxidant, anticancer, and antidiabetic effects and have been reported as characteristic constituents in several *Hedysarum* species (Altay et al. 2022; Keleke et al. 2025). The relatively higher concentration of daidzein in the roots is also consistent with the organ-specific distribution of isoflavonoids previously described in members of the genus. The comparable concentrations of genistein across the analyzed plant parts may be associated with its conserved roles in plant defense and signaling.

Quercitrin showed concentrations of 3.66 mg/g extract in leaves, 3.025 mg/g extract in stems, and 1.545 mg/g extract in roots, while acacetin showed concentrations of 1.329 mg/g extract in roots, 0.629 mg/g extract in stems, and 0.851 mg/g extract in leaves. The higher concentration of acacetin in roots may indicate organ-specific methylation patterns in flavone

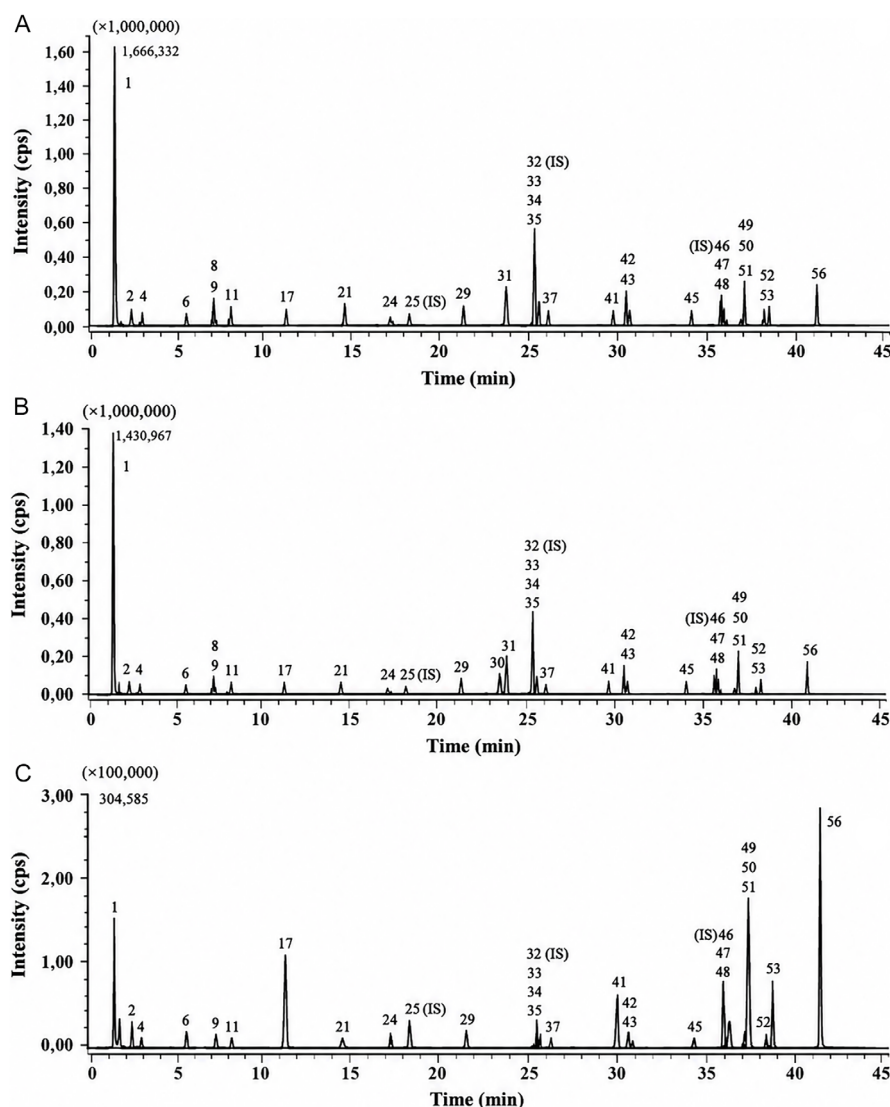


FIGURE 1 | Total ion chromatograms of the (A) leaf, (B) stalk, and (C) root extracts of HE.

biosynthesis. A similar distribution of flavone aglycones at higher concentrations in the roots has also been reported in other *Hedysarum* species, supporting the organ-dependent distribution observed in the present study (Keleke et al. 2025). Apigenin (0.114 mg/g extract in roots) and luteolin (0.025 mg/g extract in roots) were present at low concentrations; however, their reported anti-inflammatory and anticancer activities may contribute to the overall biological activity of the extracts.

Among the phenolic acids, caffeic acid showed the highest concentration in roots (0.875 mg/g extract), followed by stems (0.039 mg/g extract) and leaves (0.022 mg/g extract). Salicylic acid showed a similar distribution, with concentrations of 0.564 mg/g extract in stems, 0.453 mg/g extract in roots, and 0.231 mg/g extract in leaves. These compounds are known mediators of plant defense and are associated with antimicrobial and antioxidant properties (Altay et al. 2022). The organ-dependent variation observed for these phenolic acids agrees with previous phytochemical studies showing that hydroxycinnamic acid derivatives are distributed among different plant organs in *Hedysarum* species (Altay et al. 2022; Baltürk et al. 2025). Chlorogenic acid was present at low concentrations, with 0.122 mg/g extract

in stems, 0.057 mg/g extract in roots, and 0.03 mg/g extract in leaves. Tannic acid was present at a concentration of 0.526 mg/g extract in leaves and may contribute to the radical scavenging capacity of the extracts.

Compounds present at lower concentrations, including naringenin (0.096 mg/g extract in roots), hesperetin (0.014–0.027 mg/g extract), kaempferol (0.008–0.027 mg/g extract), and *p*-coumaric acid (≤ 0.031 mg/g extract), may also contribute to the overall phenolic composition of the extracts. Although individually minor, these metabolites are widely reported to exhibit antioxidant, antimicrobial, and enzyme inhibitory activities (Altay et al. 2022). The presence of these compounds together with the major phenolic constituents further indicates the chemical complexity of HE, which is consistent with reports describing *Hedysarum* extracts as multicomponent phytochemical systems containing multiple bioactive constituents.

A comparison of phenolic acids and flavonoid derivatives demonstrated clear organ-specific differences in the metabolic profiles of HE. Phenolic acids showed distinct variation among the analyzed plant parts, with quinic acid showing the highest

concentration in the aerial organs, while several hydroxybenzoic and hydroxycinnamic acid derivatives, such as caffeic acid, were present at relatively higher concentrations in the roots. Similarly, flavonoid derivatives were represented by a larger number of compounds and were present at higher concentrations in the aerial organs than in the roots. Overall, flavonoid glycosides constituted the major phenolic group in the aerial parts, while the roots contained a relatively higher proportion of simple phenolic acids and isoflavonoid aglycones, including acacetin and genistein. This distribution pattern is consistent with previous phytochemical investigations of *Hedysarum* species, which have reported higher concentrations of flavonoid glycosides in aerial organs and isoflavonoids in roots (Altay et al. 2022; Keleke et al. 2025). Therefore, the organ-specific phenolic composition observed in HE further supports the distinct metabolic characteristics of different plant organs and may be associated with the differences in the biological activities observed among the extracts.

Comparatively, *Hedysarum pallidum* was reported to contain kaempferol derivatives and other flavonoids, while *Hedysarum alpinum* was characterized by the presence of mangiferin and other xanthones (Dob et al. 2024; Filonova et al. 2024). Similarly, in *H. semenowii*, flavones were present at higher concentrations in the aerial parts, while isoflavones showed higher concentrations in the roots (Keleke et al. 2025). The phytochemical profile of HE generally follows this organ-specific distribution pattern but is characterized by high concentrations of isoquercitrin and quinic acid in the leaves. To the best of our knowledge, this quantitative profile has not been previously reported for the genus, providing additional information on the phytochemical characteristics of endemic *Hedysarum* species.

To investigate the phenolic metabolite profiles of leaves, stems, and roots of HE, heatmap analysis (Figure 2) and PCA (Figure 3) were performed using quantitative LC-MS/MS data. The heatmap showed organ-specific differences in metabolite concentrations. The leaf extract showed higher concentrations of flavonoid glycosides and related phenolics, including rutin, isoquercitrin, hesperidin, astragalin, nicotiflorin, miquelianin, quercetin, kaempferol, tannic acid, and hesperetin, indicating that leaves contain higher concentrations of compounds associated with antioxidant activity. In contrast, the stem extract showed higher concentrations of quinic acid, chlorogenic acid, fumaric acid, salicylic acid, and cyanoside, indicating a profile characterized by phenolic acids and organic acids. The root extract showed a composition characterized by higher concentrations of aglycone flavonoids and isoflavonoids, including acacetin, apigenin, luteolin, naringenin, daidzein, genistin, and caffeic acid, indicating that roots contain higher concentrations of these metabolites. These organ-specific patterns were also supported by the PCA biplot, in which the first two principal components represented 100.0% of the total variance, with PC1 at 73.5% and PC2 at 26.5%. The leaf extract was positioned in the positive region of PC1 and the negative region of PC2 and was associated with rutin, isoquercitrin, hesperidin, miquelianin, kaempferol, tannic acid, and galocatechin, indicating that these compounds contributed to the separation of the leaf samples. The stem extract was located in the positive region of PC2 and was associated with chlorogenic acid, salicylic acid, coumaric acid, fumaric acid, and gentisic acid, indicating a profile characterized by phenolic acids. In contrast, the root extract was positioned in the negative region of PC1 and was associated with acacetin, daidzin, genistin, apigenin, luteolin, and naringenin, indicating higher

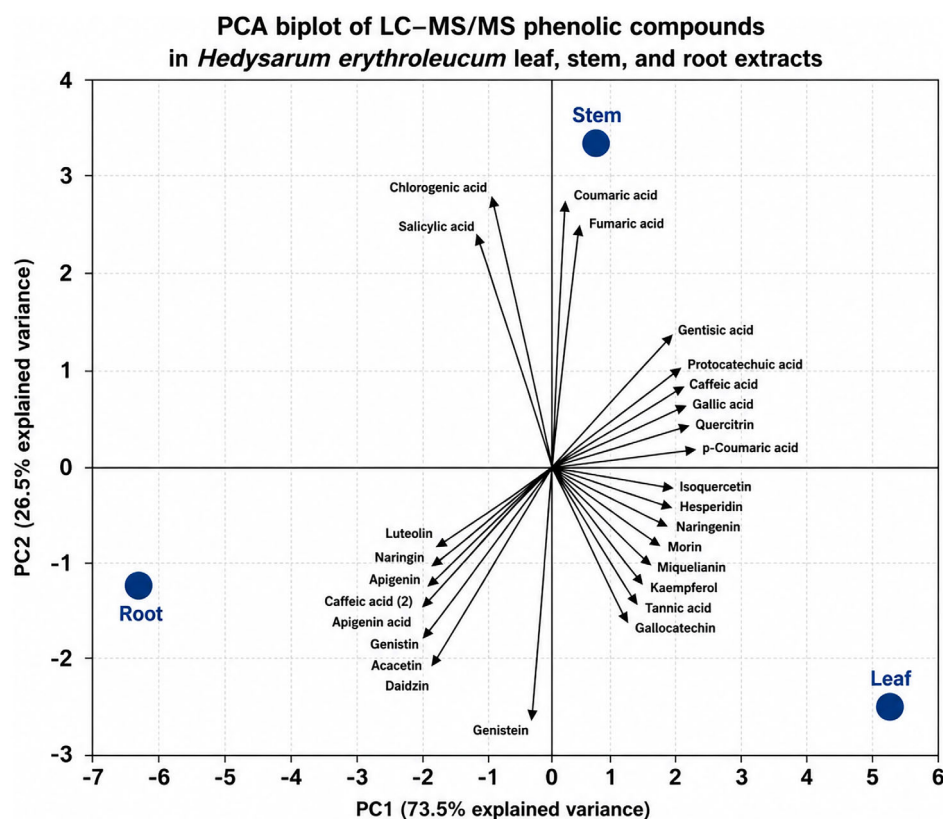


FIGURE 2 | Heatmap of LC-MS/MS-derived phenolic compounds in different organs (leaf, stem, and root) of HE.

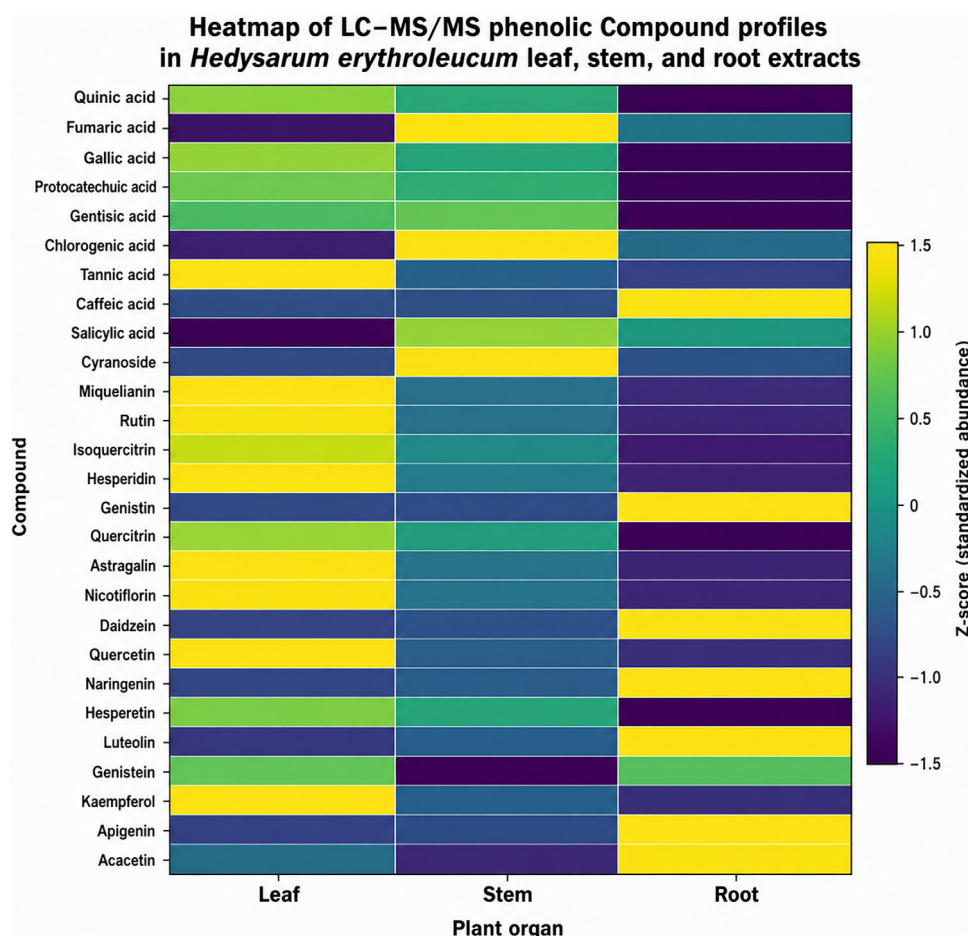


FIGURE 3 | PCA biplot of LC-MS/MS-derived phytochemicals in different organs (leaf, stem, and root) of HE.

concentrations of aglycone flavonoids and isoflavonoids in roots. These findings demonstrate that the phytochemical composition of HE is organ-dependent, with leaves containing higher concentrations of flavonoid glycosides, stems characterized by phenolic acids and organic acids and roots containing higher concentrations of aglycone flavonoids and isoflavonoids. This compositional variation may contribute to the differences observed in the biological activities of the extracts.

3.2 | Enzyme Inhibition Activity of Extracts

The inhibitory activities of leaf, stem, and root extracts of HE against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α -glycosidase were determined and compared with those of the standard inhibitors tacrine and acarbose. The findings are presented in Figures 4–6, and the corresponding IC_{50} values are presented in Table 2. The findings clearly demonstrated that enzyme inhibitory activity was strongly dependent on plant organ, with the leaf extract consistently exhibiting the highest activity against all tested enzymes.

Among the tested extracts, the leaf extract exhibited the strongest inhibitory effect against AChE with an IC_{50} value of $4.56 \pm 0.15 \mu\text{g/mL}$ ($R^2 = 0.9693$), followed by the stem ($5.21 \pm 0.18 \mu\text{g/mL}$; $R^2 = 0.9636$) and root extracts ($6.66 \pm 0.36 \mu\text{g/mL}$; $R^2 = 0.9658$). Under the same assay conditions, the reference inhibitor tacrine exhibited an IC_{50}

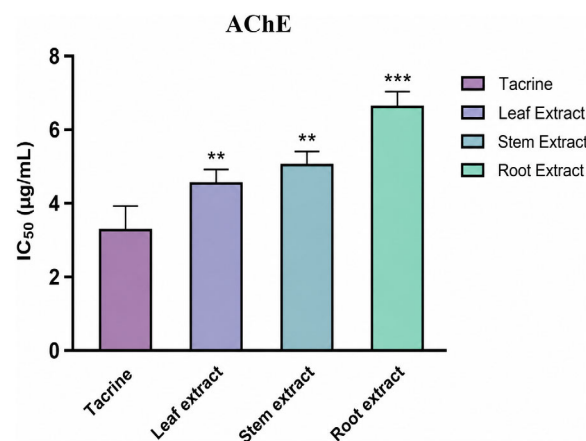


FIGURE 4 | Inhibitory values of leaf, branch, and root methanol extracts of *H. erythroleucum* on the AChE enzyme. Experiments were performed in triplicate. Statistical significance was indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. ns indicates no significant difference ($p > 0.05$).

value of $3.43 \pm 0.40 \mu\text{g/mL}$ ($R^2 = 0.9981$). Although tacrine showed lower IC_{50} values, the leaf extract also exhibited strong anticholinesterase activity. Quantitatively, the IC_{50} value of the leaf extract was 33% higher than that of tacrine, while the stem and root extracts showed 52% and 94% lower activity, respectively. This observation agrees with previous studies

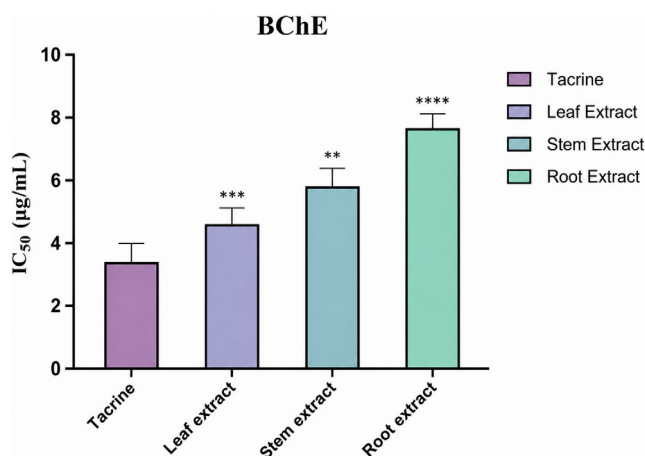


FIGURE 5 | Inhibitory values of leaf, branch, and root methanol extracts of *H. erythroleucum* on the BChE enzyme. Experiments were performed in triplicate. Statistical significance was indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. ns indicates no significant difference ($p > 0.05$).

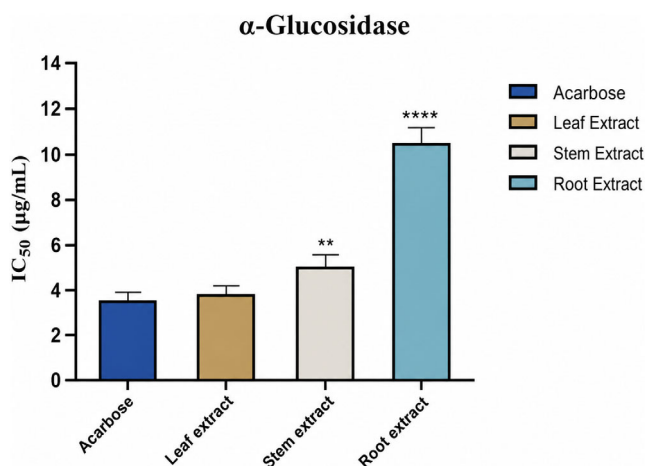


FIGURE 6 | IC₅₀ values of methanol extracts obtained from different plant parts (leaves, branches, and roots) of *H. erythroleucum* for their inhibitory effects on the α-glucosidase enzyme. Experiments were performed in triplicate. Statistical significance was indicated as follows: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$. ns indicates no significant difference ($p > 0.05$).

reporting stronger cholinesterase inhibitory activity in extracts with high flavonoid content from *Hedysarum* species (Zengin et al. 2015; Altay et al. 2022). The stronger inhibition observed

for the leaf extract suggests that HE contains constituents with promising in vitro cholinesterase inhibitory activity. However, further mechanistic investigations and in vivo studies are required to confirm their potential relevance to neurodegenerative disorders.

A similar inhibitory pattern was observed for BChE. The leaf extract again demonstrated the strongest activity with an IC₅₀ value of $4.56 \pm 0.17 \mu\text{g/mL}$ ($R^2 = 0.9859$), followed by the stem ($5.82 \pm 0.56 \mu\text{g/mL}$; $R^2 = 0.9425$) and root extracts ($7.70 \pm 0.40 \mu\text{g/mL}$; $R^2 = 0.9778$). Tacrine inhibited BChE with an IC₅₀ value of $3.18 \pm 0.20 \mu\text{g/mL}$ ($R^2 = 0.9768$). Although the inhibitory activity of the leaf extract was slightly lower than that of tacrine, the extract has significant BChE inhibitory activity. Because BChE activity increases during the progression of AD, the inhibition of this enzyme is considered pharmacologically important. Therefore, the simultaneous inhibition of AChE and BChE indicates a potentially interesting in vitro pharmacological profile; however, these observations require confirmation through mechanistic and in vivo studies before neuroprotective effects can be confirmed. The lower activities observed in stem and root extracts support the view that the concentrations of enzyme-active secondary metabolites vary among the analyzed plant parts.

The α-glycosidase inhibition results also followed the same order, with the leaf extract showing the strongest activity ($3.87 \pm 0.26 \mu\text{g/mL}$; $R^2 = 0.9991$), followed by the stem ($5.06 \pm 0.30 \mu\text{g/mL}$; $R^2 = 0.9894$) and root extracts ($10.83 \pm 0.52 \mu\text{g/mL}$; $R^2 = 0.9440$). Acarbose, used as the positive control, showed an IC₅₀ value of $3.55 \pm 0.04 \mu\text{g/mL}$ ($R^2 = 0.9992$). The inhibitory activity of the leaf extract was comparable to that of acarbose, with an IC₅₀ value 9% higher than that of the standard inhibitor. This finding is particularly important because α-glycosidase inhibitors slow carbohydrate digestion and reduce postprandial hyperglycemia, representing a major therapeutic strategy in the management of T2DM. The strong α-glycosidase inhibitory activity observed for the leaf extract suggests promising in vitro antidiabetic potential. However, additional mechanistic investigations and in vivo validation are necessary before its therapeutic relevance can be confirmed.

The high R^2 values obtained for all enzyme inhibition assays (0.9425–0.9992) indicated strong regression correlations and confirmed the reliability of the calculated IC₅₀ values. In addition, the consistent inhibitory order observed across all tested enzymes (leaf > stem > root) suggests that the differences in activity are associated with variations in phytochemical

TABLE 2 | Inhibitory effects of *Hedysarum erythroleucum* leaf, stem, and root extracts on acetylcholinesterase (AChE), butyrylcholinesterase (BChE), and α-glucosidase.

Inhibitor	IC ₅₀ , µg/mL					
	AChE	R^2	BChE	R^2	α-Glucosidase	R^2
Leaf	4.56 ± 0.15^b	0.9693	4.56 ± 0.17^b	0.9859	3.87 ± 0.26^a	0.9991
Stem	5.21 ± 0.18^c	0.9636	5.82 ± 0.56^c	0.9425	5.06 ± 0.30^b	0.9894
Root	6.66 ± 0.36^c	0.9658	7.70 ± 0.40^d	0.9778	10.83 ± 0.52^c	0.9440
Tacrine	3.43 ± 0.40^a	0.9981	3.18 ± 0.20^a	0.9768	—	—
Acarbose	—	—	—	—	3.55 ± 0.04^a	0.9992

Note: Values are expressed as mean ± SEM ($n = 3$). Different superscript letters (a–d) within the same column indicate statistically significant differences (one-way ANOVA, $p < 0.05$). R^2 represents the coefficient of determination obtained from dose–response curve analysis.

composition among the analyzed plant parts. Such variations are commonly associated with differences in the concentrations and composition of secondary metabolites among plant parts.

When compared with previous studies conducted on *Hedysarum* species, the inhibitory activities obtained in the present study were stronger. Previous investigations on members of this genus have reported moderate inhibitory effects against cholinesterases and carbohydrate-hydrolyzing enzymes, generally associated with extracts containing high concentrations of phenolic compounds and flavonoids. *H. candidissimum* was reported to exhibit acetylcholinesterase and α -glycosidase inhibitory activities, which were attributed to its high concentrations of phenolic acids and flavonoid glycosides, including chlorogenic acid derivatives and quercetin-based compounds (Altay et al. 2022). Similarly, extracts of *Hedysarum varium* showed inhibitory effects against AChE, BChE, and α -glycosidase, supporting the potential of the genus as a source of enzyme inhibitors (Zengin et al. 2015). Compared with these reports, the leaf extract of HE showed strong inhibitory activity, with IC_{50} values of 4.56 μ g/mL for both AChE and BChE and 3.87 μ g/mL for α -glycosidase, which was comparable to that of acarbose (3.55 μ g/mL).

The strong inhibitory activity observed in the leaf extract may be associated with the higher concentrations of phenolic compounds and flavonoids in photosynthetically active parts of the plant. Previous phytochemical studies on *Hedysarum* species have identified phenolic acids, flavonoid glycosides, and other polyphenolic constituents as the major metabolites contributing to biological activity. In particular, compounds such as rutin, isoquercitrin, astragalin, chlorogenic acid, and caffeic acid derivatives have been reported in several *Hedysarum* species

and are recognized as important contributors to cholinesterase and α -glycosidase inhibition (Altay et al. 2022; Baltürk et al. 2025). These phenolic constituents may interact with catalytic and accessory regions of enzymes by hydrogen bonding, hydrophobic contacts, and π - π interactions, which may reduce enzyme activity (Şöhretoğlu and Sari 2020). Accordingly, the stronger inhibitory activity of the leaf extract of HE may be related to both its higher concentration of phenolic constituents and interactions among its secondary metabolites. In contrast, the lower activities observed in the stem and root extracts may be associated with lower concentrations of these bioactive compounds or qualitative differences in metabolite composition.

To further investigate the relationship between enzyme inhibitory activity and phytochemical composition, PCA was performed by integrating the IC_{50} values for AChE, BChE, and α -glycosidase inhibition with the LC-MS/MS metabolite profiles of the leaf, stem, and root extracts of HE (Figure 7). The first two principal components represented 100.0% of the total variance, with PC1 at 79.24% and PC2 at 20.76%, indicating the separation of the plant parts according to their chemical and biological characteristics. The leaf extract was located in the negative region of both PC1 and PC2 and was associated with flavonoid glycosides and related phenolics, including isoquercitrin, hesperidin, astragalin, tannic acid, miquelianin, quercetin, and kaempferol. Because the enzyme inhibition variables, expressed as IC_{50} values, were positioned on the opposite side of the PCA plot, the leaf extract was associated with lower IC_{50} values, indicating stronger inhibitory activity against all tested enzymes. The stem extract was positioned mainly on PC2 and was associated with chlorogenic acid, salicylic acid, fumaric acid, catechin, quinic acid, and protocatechuic acid. The root extract was positioned mainly on PC1 and was associated with chlorogenic acid, salicylic acid, fumaric acid, catechin, quinic acid, and protocatechuic acid, indicating a phenolic profile with a more moderate contribution

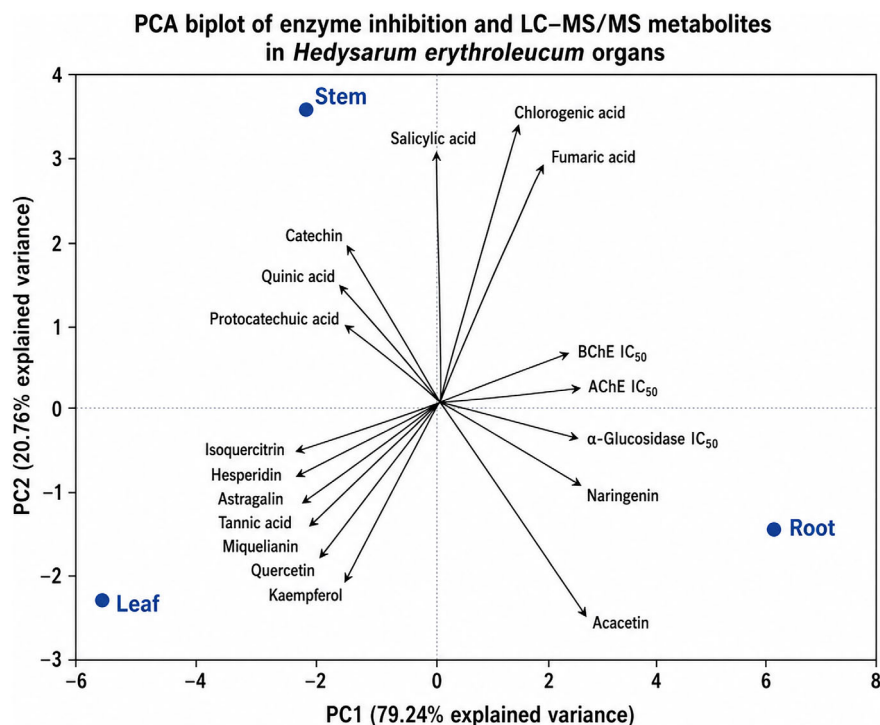


FIGURE 7 | PCA biplot integrating enzyme inhibitory activities and LC-MS/MS-derived metabolites in different organs (leaves, stems, and roots) of HE.

to enzyme inhibition. In contrast, the root extract was positioned in the positive region of PC1 and the negative region of PC2 and was associated with naringenin and acetin, as well as with the AChE, BChE, and α -glycosidase IC₅₀ variables. As higher IC₅₀ values correspond to lower inhibitory potency, this distribution indicates that the root extract showed lower enzyme inhibitory activity than the leaf extract. The PCA further indicates that the stronger enzyme inhibitory activity of the leaf extract is closely associated with the presence of flavonoid glycosides, particularly isoquercitrin, hesperidin, astragalin, miquelianin, quercetin, and kaempferol, which show an inverse association with the IC₅₀ variables. This distribution supports a close association between the organ-specific phenolic profile and enzyme inhibitory activity. The observed associations are derived from multivariate chemometric analysis and require confirmation through mechanistic investigations and compound isolation studies.

Overall, the present findings demonstrate that HE exhibits significant in vitro inhibitory activity against cholinesterase and α -glycosidase enzymes. These findings support the continued investigation of this species as a source of bioactive phenolic constituents. Further studies involving compound isolation, mechanistic investigations, and in vivo validation are required to confirm the biological relevance of these observations.

3.3 | Antioxidant Activity of Extracts

The antioxidant potential of leaf, stem, and root extracts of HE was determined using DPPH radical scavenging activity, TPC, TFC, and FRAP. The results are presented in Table 3 and show statistically significant differences among the extracts ($p < 0.05$). The findings demonstrated that antioxidant activity varied among plant parts and was associated with the concentrations of phenolic and flavonoid compounds. Among the tested extracts, the leaf extract consistently exhibited the highest antioxidant capacity in all assays, while the root extract showed the lowest activity.

The DPPH radical scavenging assay showed differences among the tested plant parts. The leaf extract exhibited the strongest radical scavenging activity with an IC₅₀ value of $9.58 \pm 0.88 \mu\text{g/mL}$, followed by the stem extract ($22.88 \pm 0.60 \mu\text{g/mL}$), while the root extract showed lower activity ($318.14 \pm 3.61 \mu\text{g/mL}$). Importantly, the antioxidant capacity of the leaf extract was highly comparable to that of standard synthetic antioxidants, including Trolox ($8.50 \pm 0.77 \mu\text{g/mL}$), BHA ($8.04 \pm 0.69 \mu\text{g/mL}$), BHT

($10.70 \pm 0.73 \mu\text{g/mL}$), and ascorbic acid ($9.91 \pm 0.87 \mu\text{g/mL}$). This observation is consistent with previous studies reporting that methanolic extracts with high phenolic content generally exhibit stronger radical scavenging activity in *Hedysarum* species (Uyar et al. 2017; Altay et al. 2022). Quantitatively, the DPPH scavenging activity of the leaf extract was 13% lower than that of Trolox, indicating activity comparable to that of the reference antioxidant. In contrast, the root extract showed 33-fold lower activity than the leaf extract, indicating major differences in phytochemical composition among plant parts.

The strong radical scavenging activity of the leaf extract may be attributed to its high phenolic and flavonoid contents. The leaf extract contained the highest total phenolic content ($119.64 \pm 1.37 \text{ mg GAE/g extract}$) and total flavonoid content ($68.87 \pm 1.02 \text{ mg QE/g extract}$), while the root extract contained lower phenolic content ($30.80 \pm 1.43 \text{ mg GAE/g extract}$) and flavonoid content ($3.33 \pm 0.95 \text{ mg QE/g extract}$). These results support the reported correlation between the phenolic content and antioxidant activity. Phenolic compounds are widely recognized for their ability to donate hydrogen atoms or electrons to reactive oxygen species and free radicals, converting them to more stable products by resonance stabilization of the resulting phenoxyl radicals (Tumilaar et al. 2024; Ahmad et al. 2025). Similarly, flavonoids can chelate transition metal ions and inhibit oxidative chain reactions, which may contribute to their antioxidant defense mechanisms (Panche et al. 2016). The higher phenolic and flavonoid contents of the leaf extract are therefore in agreement with its superior antioxidant performance observed in the present study.

The FRAP further supported the DPPH findings and demonstrated a similar activity pattern among the tested extracts. The leaf extract exhibited the highest reducing power ($175.68 \pm 0.64 \text{ mg TE/g extract}$), followed by the stem ($151.50 \pm 0.21 \text{ mg TE/g extract}$) and root extracts ($81.05 \pm 0.37 \text{ mg TE/g extract}$). Although the reducing capacities of the extracts were lower than those of synthetic standards such as ascorbic acid ($394.17 \pm 0.98 \text{ mg TE/g extract}$), BHA ($338.57 \pm 0.31 \text{ mg TE/g extract}$), and BHT ($257.80 \pm 1.24 \text{ mg TE/g extract}$), the leaf extract still demonstrated considerable electron-donating capacity. Reducing power assays are commonly used to determine the electron-donating ability of antioxidants, which reflects their capacity to reduce oxidized compounds and terminate radical chain reactions (Apak et al. 2016). Therefore, the high reducing capacity of the leaf extract indicates the presence of constituents capable of acting as

TABLE 3 | GI₅₀, IC₅₀, and tumor selectivity index (TSI) values of *Hedysarum erythroleucum* extracts against lung cancer and normal cell lines.

Extracts, $\mu\text{g/mL}$	A549		Calu-1		H1650		Beas2B		TSI
	GI ₅₀	IC ₅₀	GI ₅₀	IC ₅₀	GI ₅₀	IC ₅₀	GI ₅₀	IC ₅₀	
Leaf extract	16.35	247.23	3.27	242.72	1.14	276.95	19.32	220.77	0.86
Stem extract	11.82	234.54	4.47	228.58	1.15	131.86	3.72	226.57	1.14
Root extract	15.61	241.19	7.68	224.78	1.16	121.40	10.48	239.76	1.22
5-FU	2.30	101.63	2.08	96.37	1.81	93.14	2.50	92.71	0.96

Note: GI₅₀, concentration required to inhibit cell growth by 50%; IC₅₀, concentration required to reduce cell viability by 50%. Abbreviation: TSI, tumor selectivity index.

reductants and antioxidants (Fraga et al. 2019). Similar relationships between reducing power and phenolic composition have also been reported for other *Hedysarum* species, supporting the present findings (Altay et al. 2022; Varol et al. 2025).

When compared with previous studies conducted on *Hedysarum* species, the antioxidant activity observed for HE, particularly in the leaf extract, was generally consistent with previous reports. Previous studies have similarly demonstrated a close relationship between the phenolic content and antioxidant potential in *Hedysarum* species. In *H. candidissimum*, Altay et al. reported high levels of phenolic and flavonoid compounds, including quinic acid, chlorogenic acid, cynaroside, cosmosiin, quercetin, and luteolin, and showed that the methanol extract exhibited strong DPPH radical scavenging and reducing capacity (Altay et al. 2022). Similarly, *Hedysarum aucheri* extracts were reported to contain high phenolic and flavonoid contents, and the butanol extract, which had the highest phenolic and flavonoid contents, showed the highest antioxidant activity in CUPRAC and ABTS assays (Uyar et al. 2017). In another study, *Hedysarum cappadocicum* was analyzed together with other endemic medicinal plants, and its antioxidant activity was associated with the phenolic profile determined by RP-HPLC-DAD analysis (Bektaş et al. 2018). Compared with these reports, the leaf extract of HE showed strong radical-scavenging activity, with an IC_{50} value comparable to that of standard antioxidants. The strong antioxidant activity of the leaf extract may be associated with the higher concentrations of phenolic compounds and flavonoids in the aerial parts. This interpretation is consistent with recent findings on *Hedysarum nitidum*, in which methanol and water extracts exhibited strong antioxidant activity, and phenolic compounds such as *p*-hydroxybenzoic acid, caffeic acid, and 2-hydroxycinnamic acid were identified by HPLC (Baltürk et al. 2025).

In addition, recent studies on different plant parts of *Hedysarum* species have shown that antioxidant activity may differ among plant parts, with aerial parts generally showing stronger activity due to higher phenolic concentrations (Varol et al. 2025). Accordingly, the superior antioxidant activity of the leaf extract of HE may be associated with its higher phenolic and flavonoid contents, which can donate electrons or hydrogen atoms, neutralize free radicals, and contribute to reducing power.

The organ-dependent variation in antioxidant activity may additionally be associated with physiological and ecological factors influencing secondary metabolite biosynthesis. Leaves are continually subjected to environmental factors such as ultraviolet radiation, temperature fluctuations, and oxidative stress generated during photosynthesis. As a result, aerial parts often contain higher concentrations of antioxidant compounds as part of their protective defense mechanisms (Akula and Ravishankar 2011; Agati et al. 2012). This physiological adaptation may be associated with the higher phenolic and flavonoid contents of the leaf extract compared with the stem and root parts.

To investigate the relationship between antioxidant activity and phytochemical composition, PCA was performed by integrating the antioxidant parameters, including DPPH radical scavenging activity, FRAP, TPC, and TFC, with the LC-MS/MS metabolite profiles of the leaf, stem, and root extracts of HE (Figure 8). The PCA analysis showed the separation of the three plant parts according to their combined chemical composition and antioxidant properties. The first two principal components represented 100.0% of the total variance, with PC1 at 82.6% and PC2 at 17.4%. The leaf extract was positioned in the negative region of both PC1 and PC2 and was associated with higher TPC, TFC, reducing power, and DPPH radical scavenging activity, as well as with

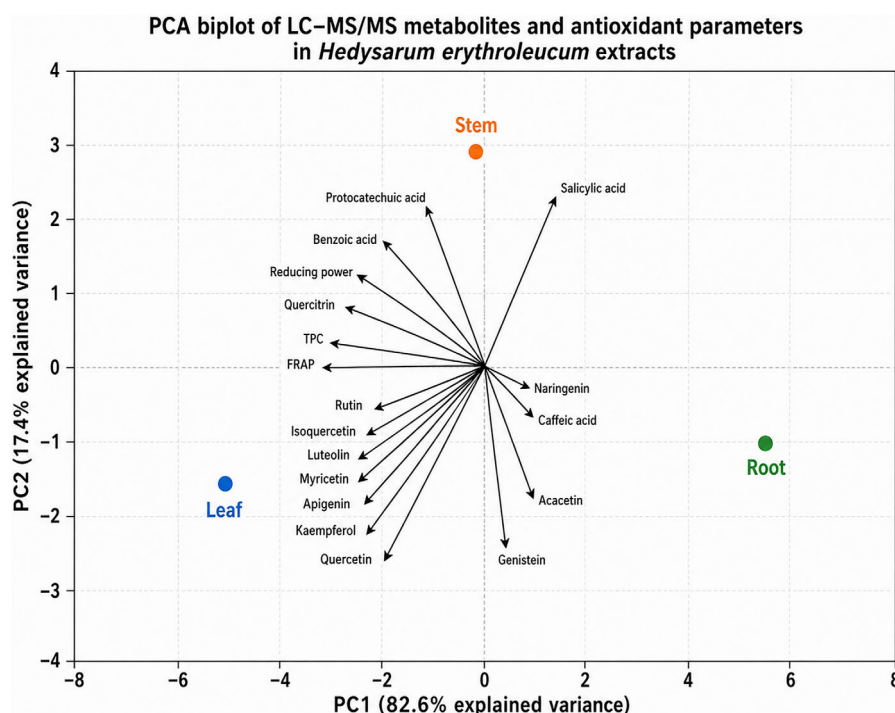


FIGURE 8 | PCA biplot integrating antiproliferative activity (GI_{50} values) and LC-MS/MS-derived metabolites in different organs (leaf, stem, and root) of HE.

several flavonoid glycosides and phenolic compounds, including isoquercitrin, astragalin, miquelianin, quercetin, kaempferol, catechin, and tannic acid. The association of these metabolites with antioxidant parameters suggests that they contribute to the strong antioxidant capacity of the leaf extract. In contrast, the stem extract was positioned mainly along PC2 and was associated with phenolic acids such as salicylic acid, protocatechuic acid, quinic acid, and catechin, indicating a phenolic composition and an intermediate antioxidant profile compared with the leaf and root extracts. The root extract was positioned in the positive region of PC1 and the negative region of PC2 and was associated with metabolites such as genistein and acacetin, together with lower TPC, TFC, and antioxidant activity. This distribution indicates that the root extract contains fewer metabolites associated with antioxidant activity than the aerial parts. The PCA results demonstrate that the higher antioxidant activity of the leaf extract is associated with higher concentrations of flavonoid glycosides and phenolic compounds, while the stem and root extracts showed different metabolite profiles associated with intermediate and lower antioxidant capacities, respectively. The close clustering of DPPH, FRAP, TPC, and TFC with flavonoid glycosides in the PCA plot further supports the association between the phenolic composition of the leaf extract and its superior antioxidant activity. These findings indicate that the antioxidant properties are associated with the combined contribution of multiple phenolic constituents instead of an individual compound.

3.4 | Antiproliferative Activity of Extracts

The antiproliferative activities of leaf, stem, and root extracts of HE against human lung cancer cell lines (A549, Calu-1, and H1650), breast cancer cell lines (MCF7 and MDA-MB-231), and normal epithelial cell lines (Beas2B and MCF10A) were determined. The antiproliferative effects were expressed as GI_{50} and IC_{50} values, and the results are presented in Tables 4 and 5. The findings demonstrated that the biological activity

of the extracts was highly dependent on both plant organ and cancer cell type.

Among the lung cancer cell lines tested, the strongest growth inhibitory activity was observed against the H1650 cell line. The leaf extract exhibited a GI_{50} value of 1.14 $\mu\text{g/mL}$, while the stem and root extracts showed GI_{50} values of 1.15 and 1.16 $\mu\text{g/mL}$, respectively. These values were comparable to those of the reference anticancer agent 5-FU, which showed a GI_{50} value of 1.81 $\mu\text{g/mL}$ under the same assay conditions. In particular, the leaf extract showed 37% stronger growth inhibitory activity than 5-FU against H1650 cells. Comparable in vitro antiproliferative effects have also been reported for extracts of other *Hedysarum* species, although differences in potency may be related to variations in phytochemical composition and the cancer cell lines used. In contrast, higher GI_{50} values were observed against A549 and Calu-1 cells, suggesting that the antiproliferative activity of the extracts may be influenced by the molecular characteristics and genetic background of the tested cancer cell lines.

The IC_{50} values obtained for lung cancer cells further supported the strong antiproliferative activity of the extracts. The stem and root extracts exhibited lower IC_{50} values against H1650 cells (131.86 and 121.40 $\mu\text{g/mL}$, respectively) compared with the leaf extract (276.95 $\mu\text{g/mL}$). These findings suggest that although the leaf extract strongly suppressed cell proliferation at low concentrations, higher concentrations may be required to induce cytotoxicity. Such differences between GI_{50} and IC_{50} profiles may indicate different mechanisms of action, including cytostatic rather than directly cytotoxic effects. A similar distinction between growth inhibition and cytotoxicity has been reported in studies using plant extracts with high phenolic content, suggesting that suppression of cell proliferation is not always associated with cell death. In addition, the comparatively moderate IC_{50} values observed against normal Beas2B cells suggest selectivity toward cancer cells, which is an important characteristic for potential anticancer agents.

TABLE 4 | GI_{50} , IC_{50} , and tumor selectivity index (TSI) values of *Hedysarum erythroleucum* extracts against breast cancer and normal cell lines.

Extracts, $\mu\text{g/mL}$	MCF7		MDA-MB-231		MCF10A		TSI
	GI_{50}	IC_{50}	GI_{50}	IC_{50}	GI_{50}	IC_{50}	
Leaf extract	17.76	151.37	8.00	174.06	1.04	136.34	0.84
Stem extract	16.49	127.21	1.58	141.00	1.00	109.85	0.82
Root extract	6.82	167.72	2.36	62.16	1.02	132.66	1.15
5-FU	1.73	96.42	1.94	92.14	1.95	93.75	0.99

Note: GI_{50} , concentration required to inhibit cell growth by 50%; IC_{50} , concentration required to reduce cell viability by 50%. Abbreviation: TSI, tumor selectivity index.

TABLE 5 | Percentage cytotoxicity of *Hedysarum erythroleucum* leaf, stem, and root extracts against lung, breast cancer, and normal cell lines.

Cytotoxicity, %	A549	Calu-1	H1650	Beas2B	MCF-7	MDA-MB-231	MCF-10A
Leaf extract	6.73	3.54	3.99	5.93	5.46	6.10	4.36
Stem extract	6.72	4.08	5.06	4.57	6.07	6.84	6.50
Root extract	5.78	5.86	6.81	6.31	6.93	12.47	5.66
5-FU	6.69	12.70	9.97	6.60	10.29	8.94	8.63

Note: Values are expressed as percentage cytotoxicity determined by the LDH release assay.

The TSI values provided additional insight into the selectivity of the extracts for cancer cells relative to normal cells. In lung cancer models, the root extract exhibited the highest selectivity (1.22), followed by the stem (1.14) and leaf extracts (0.86). Interestingly, the root extract showed a higher selectivity index than 5-FU (0.96), suggesting a more favorable biological profile for normal epithelial cells. Selective cytotoxicity is considered one of the most critical properties for the development of novel anticancer therapeutics because it reduces the effects on healthy tissues during treatment. Although the selectivity indices were moderate, similar values have been reported for crude plant extracts in previous *Hedysarum* studies, indicating that additional purification may improve biological selectivity (Uyar et al. 2017).

The antiproliferative activities of the extracts against breast cancer cell lines representing different molecular characteristics were also determined. Among the tested samples, the root extract showed the strongest activity against MCF7 cells with a GI_{50} value of 6.82 $\mu\text{g/mL}$, while the stem extract showed the strongest activity against the MDA-MB-231 cell line with a GI_{50} value of 1.58 $\mu\text{g/mL}$. The stem extract showed slightly stronger antiproliferative activity against MDA-MB-231 cells than 5-FU, which showed a GI_{50} value of 1.94 $\mu\text{g/mL}$, highlighting its promising in vitro antiproliferative activity. Triple-negative breast cancer is associated with high metastatic potential and limited treatment options; therefore, the activity observed against MDA-MB-231 cells is of particular interest. This observation is also consistent with previous reports demonstrating the sensitivity of triple-negative breast cancer cells to plant extracts with high flavonoid content.

The IC_{50} profiles in breast cancer cell lines showed differences among the extracts. The root extract exhibited the lowest IC_{50} value against MDA-MB-231 cells (62.16 $\mu\text{g/mL}$), indicating a stronger cytotoxic effect than the leaf and stem extracts. This result suggests that root-derived metabolites may contain compounds with stronger apoptosis-inducing or membrane-disruptive capacities. In contrast, the stem extract showed the lowest IC_{50} value against MCF7 cells (127.21 $\mu\text{g/mL}$), suggesting differences in sensitivity between MCF7 and MDA-MB-231 breast cancer cells. Such variations may be associated with differences in hormone receptor expression, oxidative stress response pathways, mitochondrial metabolism, and intracellular signaling mechanisms among the tested cell lines.

The TSI values obtained for breast cancer cell lines showed that the stem extract had the highest selectivity for cancer cells (0.82), followed by the leaf extract (0.84), while the root extract showed lower selectivity (1.15). Although the selectivity values were similar to those of 5-FU (0.99), the findings indicate that certain extracts of HE may show antiproliferative effects with limited effects on normal mammary epithelial cells. These findings support the continued investigation of this species as a potential source of bioactive compounds for anticancer research. However, compound isolation, mechanistic studies, and in vivo validation are required before therapeutic applications can be considered.

When compared with previous studies conducted on *Hedysarum* species, the antiproliferative activity observed in the present study was strong, particularly against H1650 and MDA-MB-231 cancer cell lines. Previous reports support the cytotoxic

and antiproliferative potential of the genus *Hedysarum*. The *H. aucheri* extracts were tested against several cancer cell lines, including A549 and MCF-7, and the dichloromethane extract showed strong cytotoxic activity, with IC_{50} values between 0.64 and 24 $\mu\text{g/mL}$, while showing lower toxicity in normal HEK293 cells (Uyar et al. 2017). Similarly, *H. candidissimum* was reported to show cytotoxic effects against HT-29 and MDA-MB-453 cancer cell lines, and this activity was associated with its phytochemical profile containing high concentrations of phenolic compounds and flavonoids (Altay et al. 2022). In addition, prenylated chalcones isolated from the roots of *Hedysarum gmelinii* showed moderate antiproliferative activity against several human cancer cell lines, including A549, HepG2, DU145, BGC823, and HCT116 (Liu et al. 2018). Compared with these studies, the low GI_{50} values obtained for HE, especially in H1650 and MDA-MB-231 cells, indicate that this species has a strong antiproliferative profile within the genus.

The antiproliferative activity of HE may be related to the phenolic and flavonoid composition of the extracts. Previous phytochemical studies on *Hedysarum* species have reported phenolic acids, flavonoids, chalcones, and other polyphenolic compounds as important bioactive constituents of the genus (Liu et al. 2018; Altay et al. 2022). In particular, prenylated chalcones isolated from *H. gmelinii* were shown to exhibit significant antiproliferative effects against cancer cell lines, confirming the contribution of these compounds to the cytotoxic potential of the genus (Liu et al. 2018). These compounds are known to inhibit cancer cell proliferation by regulating oxidative stress, inducing apoptosis, disrupting mitochondrial function, and modulating cell cycle-related pathways (Abotaleb et al. 2018). Accordingly, the strong growth inhibitory effects observed in the present study may result from the combined or synergistic action of multiple secondary metabolites instead of a single compound.

The organ-dependent variation observed in antiproliferative activity may additionally reflect differences in the biosynthesis and concentrations of secondary metabolites among plant parts. Leaves generally contain higher concentrations of phenolic antioxidants and flavonoid glycosides, while roots contain higher concentrations of lipophilic compounds, aglycone flavonoids, and stress-related metabolites. Such differences in phytochemical composition may be associated with the variations observed between growth inhibitory and cytotoxic effects among the extracts. In particular, the stronger cytotoxicity of root extracts against certain cancer cell lines may suggest the presence of more membrane-active or apoptosis-inducing metabolites in roots.

To investigate the relationship between antiproliferative activity and phytochemical composition, PCA was performed by integrating the GI_{50} values obtained from lung (A549, Calu-1, and H1650) and breast (MCF-7 and MDA-MB-231) cancer cell lines with the LC-MS/MS metabolite profiles of the leaf, stem, and root extracts of HE (Figure 9). The first two principal components represented 100.0% of the total variance, with PC1 at 72.63% and PC2 at 27.37%, indicating the separation of the three plant parts according to their combined chemical composition and antiproliferative activity. The leaf extract was positioned in the negative region of both PC1 and PC2 and was

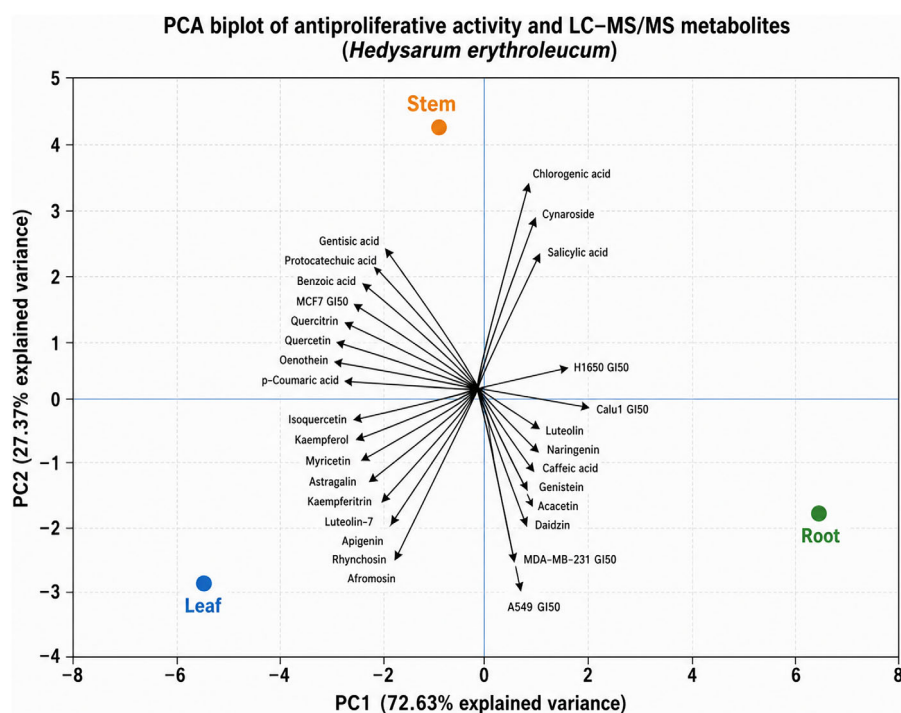


FIGURE 9 | PCA biplot integrating antioxidant parameters and LC-MS/MS-derived metabolites in different organs (leaf, stem, and root) of HE.

associated with several flavonoid glycosides and related phenolic compounds, including isoquercitrin, astragaloside, miquelianin, quercetin, kaempferol, hesperidin, and tannic acid. These compounds have been reported to show antiproliferative, proapoptotic, and cell cycle-modulating effects in a variety of cancer models (Roohbakhsh et al. 2015; Tajik et al. 2017; Reyes-Farias and Carrasco-Pozo 2019), and their high concentrations in the leaf extract suggest that they may contribute to the cytotoxic activity observed against both lung and breast cancer cell lines. In contrast, the stem extract was positioned mainly along PC2 and was associated with chlorogenic acid, quinic acid, fumaric acid, salicylic acid, and catechin. Although these metabolites are associated with antioxidant and moderate antiproliferative properties, their contribution to cytotoxic activity may be lower than that of the flavonoid profile identified in the leaf extract. The root extract was positioned in the positive region of PC1 and the negative region of PC2 and was associated with aglycone flavonoids and isoflavonoids, particularly naringenin, acacetin, genistein, and glycitin. These metabolites have previously been reported to exhibit anticancer effects by inducing apoptosis and regulating signaling pathways; however, the lower concentrations of flavonoid glycosides in the root extract may be associated with its antiproliferative profile compared with the aerial parts. The PCA further demonstrates that the antiproliferative responses are associated with the organ-specific distribution of phenolic constituents identified by LC-MS/MS. The close positioning of flavonoid glycosides and selected aglycone flavonoids relative to the leaf and root extracts suggests that these metabolites may collectively contribute to the observed antiproliferative activity. However, as the present study did not include compound isolation or molecular mechanism analyses, these associations should be considered chemometric relationships that require further validation.

3.5 | Cytotoxicity Analysis

The cytotoxic effects of HE extracts were further assessed using the LDH release assay, which determines the activity of lactate dehydrogenase released from cells with compromised membrane integrity into the extracellular environment and is widely used as an indicator of membrane integrity and cellular toxicity. As presented in Table 6, all extracts exhibited generally low cytotoxicity across the tested cell lines, with values between 3.54% and 12.47%. In lung cancer cell lines (A549, Calu-1, and H1650), cytotoxicity values were between 3.54% and 6.81% and were lower than or comparable to those observed for 5-FU, particularly in Calu-1 and H1650 cells. Similarly, normal lung epithelial cells (Beas2B) showed low cytotoxic responses (4.57%–6.31%), indicating limited membrane-associated toxicity in healthy lung cells. In breast cancer models, slightly higher cytotoxicity was observed, especially in MDA-MB-231 cells, where the root extract produced the highest value (12.47%), while MCF7 cells exhibited moderate responses between 5.46% and 6.93%. Normal mammary epithelial cells (MCF10A) also showed low cytotoxicity (4.36%–6.50%), with all values lower than that of 5-FU (8.63%). When considered together with the MTT assay results, these findings indicate that the extracts reduce cancer cell proliferation while showing relatively low toxicity in noncancerous epithelial cells, resulting in moderate tumor selectivity. A similar relationship between growth inhibition and limited LDH release has been reported for plant extracts with high phenolic content, suggesting that suppression of cell proliferation is not always associated with marked membrane damage. In addition, the low LDH release observed in samples with significant antiproliferative activity suggests that the extracts may act mainly by inducing cytostatic mechanisms, such as apoptosis induction, oxidative stress modulation, and interference with cancer-related signaling pathways, without inducing direct necrotic membrane damage. These observations are also consistent with previous studies on

TABLE 6 | Total phenolic content, total flavonoid content, and antioxidant activities of *Hedysarum erythroleucum* leaf, stem, and root extracts.

Sample	DPPH [•] scavenging IC ₅₀ , µg/mL	Total phenolic, mg GAE/g extract	Total flavonoids, mg QE/g extract	Reducing power, mg TE/g extract
Leaf extract	9.58 ± 0.88 ^{a,b}	119.64 ± 1.37 ^a	68.87 ± 1.02 ^a	175.68 ± 0.64 ^d
Stem extract	22.88 ± 0.60 ^c	86.21 ± 1.35 ^b	51.73 ± 1.00 ^b	151.50 ± 0.21 ^e
Root extract	318.14 ± 3.6 ^d	30.80 ± 1.43 ^c	3.33 ± 0.95 ^c	81.05 ± 0.37 ^f
Trolox	8.50 ± 0.77 ^a	—	—	—
BHA	8.04 ± 0.69 ^a	—	—	338.57 ± 0.31 ^b
BHT	10.70 ± 0.73 ^b	—	—	257.80 ± 1.24 ^c
Ascorbic acid	9.91 ± 0.87 ^{a,b}	—	—	394.17 ± 0.98 ^a

Note: Values are expressed as mean ± SEM (n = 3). Different superscript letters (a–f) within the same column indicate statistically significant differences (one-way ANOVA, *p* < 0.05).

Hedysarum species reporting that phenolic constituents can inhibit cancer cell growth while producing relatively low cytotoxic effects in normal cells (Uyar et al. 2017; Altay et al. 2022).

4 | Conclusion

This study comprehensively investigated the phytochemical composition and biological activities of methanolic extracts obtained from the leaf, stem, and root of HE and demonstrated organ-dependent differences in both metabolite composition and biological activity. LC-MS/MS analysis showed that the leaf extract contained high concentrations of flavonoid glycosides, including isoquercitrin, astragalin, rutin, hesperidin, and miquelianin, while the stem extract contained higher concentrations of quinic acid and catechin derivatives, and the root extract was characterized by a higher proportion of aglycone flavonoids and isoflavonoids. These phytochemical differences were associated with the biological activities, with the leaf extract consistently showing the strongest antioxidant activity, the highest total phenolic and flavonoid contents, and the strongest inhibitory effects against AChE, BChE, and α -glycosidase, with IC₅₀ values comparable to those of standard inhibitors. The extracts also showed antiproliferative activity against lung and breast cancer cell lines, particularly H1650 and MDA-MB-231, while showing low cytotoxicity in normal epithelial cells and TSI values comparable to those of the reference compound. The low LDH-based cytotoxicity in samples showing significant antiproliferative effects suggests a mainly cytostatic mode of action. These findings indicate that *Hedysarum erythroleucum*, particularly the leaf extract, contains a diverse phenolic profile and exhibits significant antioxidant, enzyme inhibitory, and antiproliferative activities. However, as the present study was performed using crude methanolic extracts and in vitro biological assays, the observed activities should not be attributed directly to individual phytochemical constituents or considered conclusive for therapeutic applications. Future studies should focus on the isolation and characterization of the active constituents, bioactivity-guided fractionation, mechanistic investigations, and in vivo validation to confirm the pharmacological potential and safety of these extracts.

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Conflicts of Interest

The authors declare that they have no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available within the article.

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