

Phytochemical Characterization and Bioactivity of *Genista unalii*, a Recently Described Endemic Species from Türkiye

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Abstract

Genista unalii M. Dinç & Y. Bağcı (Fabaceae) is a recently described endemic species restricted to the Bolkar Mountains of Türkiye. Despite its taxonomic affinity to pharmacologically important *Genista* species, its phytochemical composition and biological properties have remained unexplored. This study aimed to characterize the phenolic profile of *G. unalii* aerial parts and evaluate the antioxidant and enzyme inhibitory activities of ultrasonic-assisted ethyl acetate, methanol, and water extracts.

Phenolic constituents were characterized by LC–MS/MS using authentic reference standards, while antioxidant capacity was assessed by six complementary in vitro assays. Enzyme inhibitory activities against acetylcholinesterase, butyrylcholinesterase, tyrosinase, α -amylase, and α -glucosidase were determined, and relationships between phytochemical composition and biological activities were investigated using Pearson correlation and hierarchical clustering analyses.

Twenty phenolic compounds were identified, with distinct solvent-dependent phytochemical profiles among the extracts. The methanol extract exhibited the most balanced antioxidant performance and showed the strongest α -glucosidase inhibitory activity ($IC_{50} = 0.72$ mg/mL), exceeding that of the reference inhibitor acarbose ($IC_{50} = 0.97$ mg/mL). Correlation analysis indicated that flavonoid-rich fractions, particularly those containing apigenin and eriodictyol, were closely associated with antioxidant activity and α -glucosidase inhibition.

This study provides the first comprehensive phytochemical and biological characterization of *G. unalii*, identifies the species as a promising source of bioactive phenolics with antioxidant and antidiabetic potential, and contributes valuable chemotaxonomic information for endemic Turkish representatives of the genus *Genista*.

Introduction

The genus *Genista* L. (Fabaceae) comprises numerous species distributed throughout the Mediterranean region and western Asia, with Türkiye representing an important centre of diversity and endemism. *Genista unalii* M. Dinç & Y. Bağcı, a recently described species belonging to *Genista* sect. *Spartocarpus* Spach, was formally recognized in 2018 based on a unique combination of morphological characters, including leaf morphology, stipule persistence, pod shape, and inflorescence architecture (Dinç and Bağcı 2018). The species is restricted to limestone slopes of the Bolkar Mountains in southern Türkiye, where it occurs within a narrow altitudinal range of approximately 1,500–1,700 m. Its closest morphological relatives, *G. januensis* subsp. *lydia* and *G. lobelii*, have been reported to contain diverse phenolic constituents with remarkable biological activities (Boukaabache et al. 2024; Argon et al. 2025). Nevertheless, no phytochemical or biological information has been available for *G. unalii* since its description.

Species of *Genista* have long attracted attention because of their ethnobotanical importance and rich secondary metabolite composition. Traditional uses reported from Türkiye and neighbouring

Mediterranean regions include the treatment of diabetes, rheumatic disorders, urinary infections, and inflammatory conditions (Rauter et al. 2009). Phytochemical investigations have demonstrated that flavonoids, phenolic acids, and related phenylpropanoids constitute the major bioactive metabolites of the genus and are largely responsible for its antioxidant, antidiabetic, antimicrobial, and enzyme inhibitory properties (Pustahija et al. 2023; Argon et al. 2025). Despite the increasing number of phytochemical studies on *Genista*, recently described endemic species remain largely unexplored, limiting our understanding of both their biological potential and the phytochemical diversity within the genus.

Beyond their pharmacological relevance, secondary metabolites serve as valuable chemotaxonomic markers that complement morphological evidence and contribute to understanding evolutionary relationships within Fabaceae (Wink 2013). Within the genus *Genista*, comparative analyses of phenolic profiles have increasingly been recognized as informative for evaluating phytochemical similarities among closely related taxa and may provide complementary evidence for taxonomic interpretation (Pustahija et al. 2023; Boukaabache et al. 2024; Argon et al. 2025). Therefore, characterizing the metabolite composition of newly described endemic species not only facilitates the identification of biologically active compounds but also expands the chemotaxonomic knowledge of the genus.

Ultrasonic-assisted extraction (UAE), combined with LC–MS/MS analysis, provides an efficient and reliable approach for the recovery and characterization of phenolic compounds from plant materials (Chemat et al. 2017; Cittan and Çelik 2018). This analytical strategy facilitates comparative phytochemical investigations and has been increasingly applied to studies of *Genista* and other phenolic-rich taxa (Pustahija et al. 2023; Argon et al. 2025).

The present study aimed to characterize the phytochemical composition and biological potential of *G. unalii* aerial parts. Specifically, ultrasonic-assisted ethyl acetate, methanol, and water extracts were prepared and analysed by LC–MS/MS for phenolic profiling, followed by the determination of total phenolic and flavonoid contents, evaluation of antioxidant and enzyme inhibitory activities, and investigation of the relationships between phytochemical composition and biological activities using correlation and hierarchical clustering analyses. To the best of our knowledge, this study represents the first comprehensive phytochemical and biological characterization of *G. unalii*, providing a valuable foundation for future phytochemical, chemotaxonomic, and pharmacological investigations of endemic Turkish *Genista* species.

Materials and Methods

Plant Material

Aerial parts of *Genista unalii* M. Dinç & Y. Bağcı (Fabaceae) were collected during the flowering period in June 2025 from natural populations in the vicinity of Sertavul Pass, located between Mersin and Karaman provinces in the Anatolian region of Turkey. The botanical identification of the plant material

was performed by Prof. Dr. Muhittin Dinç from the Ahmet Keleşoğlu Faculty of Education, Necmettin Erbakan University, and a voucher specimen was deposited in the herbarium under the accession number M. Dinç 3689 for future reference. The collected aerial parts (stems, leaves, and flowers) were thoroughly cleaned to remove soil and debris, then air-dried in the shade at room temperature (20–25°C) for approximately 10–14 days until constant weight was achieved. The dried plant material was ground into a fine powder using a mechanical grinder and passed through a 40-mesh sieve to ensure particle size uniformity. The powdered material was stored in airtight amber glass containers at room temperature in a dark, dry place until extraction.

Extraction Procedures

Three extracts were prepared from the aerial parts of *Genista unalii* using ethyl acetate, methanol, and distilled water as extraction solvents. Ultrasonic-assisted extraction (UAE) was performed under identical conditions for all solvents.

Powdered aerial parts of *G. unalii* (10 g) were extracted separately with 100 mL of ethyl acetate ($\geq 99.5\%$, GC grade), methanol ($\geq 99.9\%$, HPLC grade), or distilled water at a plant-to-solvent ratio of 1:10 (w/v). The extraction was carried out in an ultrasonic bath (40 kHz, 300 W) at 30°C for 30 min and repeated twice under the same conditions. The combined extracts were filtered through Whatman No. 1 filter paper.

The ethyl acetate and methanol filtrates were concentrated under reduced pressure at 40°C using a rotary evaporator, whereas the aqueous filtrate was frozen at -18°C and subsequently freeze-dried (lyophilized). The resulting dry extracts were weighed to determine extraction yields and stored at -20°C in airtight amber glass vials until further phytochemical and biological analyses.

Determination of Total Phenolic and Flavonoid Contents

Total phenolic content (TPC) and total flavonoid content (TFC) were determined using the Folin–Ciocalteu colorimetric assay (Singleton and Rossi 1965) and the aluminum chloride colorimetric method (Chang et al. 2002), respectively, with slight modifications (Sarikurkcu C et al. 2015; Arumugam et al. 2019; Sarikurkcu Cengiz et al. 2020; Karakus Z. 2026c, 2026b). Gallic acid and rutin were used as calibration standards for TPC and TFC, respectively, and the results were expressed as milligrams of gallic acid equivalents (mg GAE/g extract) and rutin equivalents (mg RE/g extract). All measurements were performed in triplicate. Detailed experimental procedures are provided in the Supplementary Material.

LC-MS/MS Analysis

Phenolic compounds were identified and quantified by LC–ESI–MS/MS according to the validated method of Cittan and Çelik (2018). Analyses were performed using an Agilent 1260 Infinity liquid chromatography system coupled to an Agilent 6420 Triple Quad mass spectrometer (Agilent

Technologies, Santa Clara, CA, USA). Chromatographic separation was achieved on a Poroshell 120 EC-C18 column (100 × 4.6 mm, 2.7 µm) maintained at 25°C. The mobile phase consisted of solvent A (0.1% v/v formic acid in water) and solvent B (methanol), using the following gradient program: 0–3 min, 2% B; 6 min, 25% B; 10 min, 50% B; 14–17 min, 95% B; and 17.5 min, 2% B. The flow rate was 0.4 mL/min, and the injection volume was 2 µL.

Mass spectrometric detection was performed using an electrospray ionization (ESI) source operated in both positive and negative multiple reaction monitoring (MRM) modes, depending on the analyte. The optimized source parameters were as follows: capillary voltage, – 3.5 kV; gas temperature, 300°C; gas flow, 11 L/min; and nebulizer pressure, 40 psi. Compound identification and quantification were based on retention times and optimized MRM transitions of authentic standards. A total of 31 phenolic compounds were targeted for analysis, and the corresponding MRM transitions and analytical parameters are provided in Supplementary Table S1.

Antioxidant Activity Assays

The antioxidant capacities of *G. unalii* extracts were evaluated using six complementary in vitro assays representing different mechanisms of antioxidant action: phosphomolybdenum total antioxidant capacity (Prieto et al. 1999), DPPH radical scavenging (Brand-Williams et al. 1995), ABTS radical scavenging (Re et al. 1999), CUPRAC reducing power (Apak et al. 2006), FRAP reducing power (Benzie and Strain 1996), and ferrous ion chelating activity (Dinis et al. 1994), with slight modifications (Sarikurkcü C et al. 2016; Sarikurkcü Cengiz et al. 2020; Karakus Z. 2026a; Karakus Z and Sarikurkcü 2026). Trolox served as the positive control for all assays except metal chelation, where EDTA was used. Results were expressed as IC₅₀/EC₅₀ values and as standard-equivalent activities. Detailed experimental procedures are provided in the Supplementary Material.

Relative Antioxidant Capacity Index (RACI)

Prior to Relative Antioxidant Capacity Index calculation, antioxidant assay results expressed as IC₅₀ or EC₅₀ values were adjusted so that lower values corresponded to higher antioxidant capacity, ensuring consistency in data standardization. The RACI was calculated to enable an integrated comparison of antioxidant activities obtained from different assays, as previously described (Sun and Tanumihardjo 2007; Petrovic et al. 2016). RACI values were obtained by standardizing individual antioxidant assay results based on their mean and standard deviation and then averaging the standardized scores for each extract.

Enzyme Inhibition Assays

The enzyme inhibitory activities of *G. unalii* extracts were evaluated using well-established spectrophotometric assays: acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibition

(Ellman et al. 1961), tyrosinase inhibition (Masamoto et al. 2003), α -amylase inhibition (Xiao et al. 2006), and α -glucosidase inhibition (Kim et al. 2005), with slight modifications (Popović-Djordjević et al. 2019; Karakus Z and Sarikurkcü 2026). For all enzyme inhibition assays, percent inhibition was calculated at various extract concentrations, and IC_{50} values (concentration required to inhibit 50% of enzyme activity) were determined by plotting percent inhibition against extract concentration. Detailed experimental procedures are provided in the Supplementary Material.

Statistical Analysis

All experiments were performed in triplicate, and the results are presented as mean $\pm$ standard deviation (SD). Statistical analyses were carried out using IBM SPSS Statistics version 22.0 (IBM Corp., Armonk, NY, USA). Differences among the ethyl acetate, methanol, and water extracts were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test. Differences were considered statistically significant at $p < 0.05$, and different superscript letters in the tables indicate significant differences among extracts.

Pearson correlation analysis was performed to investigate the relationships among total phenolic content, total flavonoid content, individual phenolic compounds, antioxidant activities, and enzyme inhibitory activities. To visualize the overall association patterns, hierarchical cluster analysis (HCA) was performed using the average linkage (UPGMA) method based on Pearson correlation distances ($1 - r$). The clustered correlation heatmap was generated to identify groups of variables exhibiting similar correlation patterns.

Use of Artificial Intelligence

The authors declare that artificial intelligence–assisted tools were used solely for language editing and grammar checking to improve clarity and readability of the manuscript. No artificial intelligence tool was used for data generation, data analysis, interpretation of results, or scientific decision-making. All scientific content, interpretations, and conclusions were generated by the authors, who take full responsibility for the integrity and originality of the work.

Results

3.1. Extraction yields and total phenolic and flavonoid contents

Ultrasonic-assisted extraction of the aerial parts of *G. unalii* with ethyl acetate, methanol, and water afforded dry extract yields of 2.48%, 6.46%, and 5.50%, respectively. The extraction yields, total phenolic content (TPC), and total flavonoid content (TFC) are summarized in Table 1.

Table 1
Extraction yield, total phenolic content and total flavonoid content of extracts from *G. unalii*

Extract	Total phenolic (mg GAE/g extract)	Total flavonoid (mg RE/g extract)
Ethyl acetate	51.87 ± 0.31 ^a	19.52 ± 0.09 ^b
Methanol	34.91 ± 1.00 ^b	38.42 ± 1.28 ^a
Water	28.12 ± 0.62 ^c	1.19 ± 0.04 ^c
Values are expressed as mean ± SD (n = 3). Different superscript italic letters within the same column indicate significant differences among extracts according to one-way ANOVA followed by Tukey's HSD test (<i>p</i> < 0.05). GAE, gallic acid equivalent; RE, rutin equivalent.		

The ethyl acetate extract exhibited the highest TPC (51.87 ± 0.31 mg GAE/g extract), followed by the methanol (34.91 ± 1.00 mg GAE/g extract) and water (28.12 ± 0.62 mg GAE/g extract) extracts, with significant differences among all extracts (*p* < 0.05). In contrast, the methanol extract showed the highest TFC (38.42 ± 1.28 mg RE/g extract), significantly exceeding the ethyl acetate (19.52 ± 0.09 mg RE/g extract) and water (1.19 ± 0.04 mg RE/g extract) extracts (*p* < 0.05).

3.2. Phenolic compound profile by LC–MS/MS

A total of 20 phenolic compounds, comprising 11 phenolic acids and 9 flavonoids, were identified by LC–MS/MS analysis in the ethyl acetate, methanol, and water extracts of *Genista unalii*. Representative LC–MS/MS multiple reaction monitoring (MRM) chromatograms are presented in Fig. 1, whereas the quantitative composition of the extracts is summarized in Table 2. The quantitative distribution of the identified compounds differed markedly among the extracts.

Table 2
Phenolic compounds identified and quantified in extracts from *G. unalii* by LC–MS/MS analysis (µg/g extract).

No	Compounds	Ethyl acetate	Methanol	Water
1	Ferulic acid	104 ± 3 ^a	202 ± 2 ^a	39.8 ± 0.4 ^a
2	Chlorogenic acid	33.4 ± 0.2 ^a	153 ± 1 ^a	3.32 ± 0.08 ^a
3	Eriodictyol	128 ± 3 ^a	111 ± 1 ^a	nd
4	Apigenin	159 ± 3 ^a	87.7 ± 2.9 ^a	nd
5	3-Hydroxybenzoic acid	58.3 ± 2.1 ^a	58.6 ± 0.1 ^a	57.6 ± 0.3 ^a
6	4-Hydroxybenzoic acid	58.3 ± 1.9 ^a	58.7 ± 0.5 ^a	57.2 ± 0.7 ^a
7	Luteolin	40.9 ± 0.3 ^a	49.4 ± 0.5 ^a	1.35 ± 0.04 ^a
8	Vanillin	59.5 ± 0.9 ^a	45.3 ± 0.1 ^a	34.7 ± 0.4 ^a
9	Apigenin 7-glucoside	25.1 ± 0.4 ^a	43.2 ± 0.6 ^a	1.43 ± 0.01 ^a
10	Luteolin 7-glucoside	24.4 ± 0.6 ^a	38.5 ± 0.8 ^a	1.42 ± 0.01 ^a
11	<i>p</i> -Coumaric acid	37.0 ± 0.5 ^a	27.8 ± 0.1 ^a	23.3 ± 0.1 ^a
12	Quercetin	41.1 ± 1.0 ^a	25.7 ± 0.1 ^a	nd
13	Syringic acid	7.30 ± 0.25 ^a	15.5 ± 0.5 ^a	93.6 ± 0.3 ^a
14	Hyperoside	5.06 ± 0.13 ^a	13.4 ± 0.3 ^a	1.83 ± 0.01 ^a
15	Kaempferol	66.0 ± 4.0 ^a	13.0 ± 1.1 ^a	nd
16	Protocatechuic acid	9.40 ± 0.16 ^a	10.3 ± 0.4 ^a	nd
17	Caffeic acid	7.55 ± 0.12 ^a	6.07 ± 0.33 ^a	0.71 ± 0.01 ^a
18	Rosmarinic acid	16.6 ± 0.4 ^a	4.61 ± 0.19 ^a	3.04 ± 0.07 ^a
19	Sinapic acid	3.32 ± 0.37 ^a	4.42 ± 0.31 ^a	nd
20	Verbascoside	5.29 ± 0.06 ^a	2.43 ± 0.04 ^a	nd
21	(-)-Epicatechin	nd	nd	nd
22	(+) -Catechin	nd	nd	nd
23	2-Hydroxycinnamic acid	nd	nd	nd
24	3,4-Dihydroxyphenylacetic acid	nd	nd	nd

Values are expressed as mean ± SD (n = 3). Different superscript italic letters within the same row indicate significant differences among extracts according to one-way ANOVA followed by Tukey's HSD test (*p* < 0.05). nd, not detected.

No	Compounds	Ethyl acetate	Methanol	Water
25	Gallic acid	nd	nd	nd
26	Hesperidin	nd	nd	nd
27	Taxifolin	nd	nd	nd
Values are expressed as mean $\pm$ SD (n = 3). Different superscript italic letters within the same row indicate significant differences among extracts according to one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). nd, not detected.				

The ethyl acetate extract was characterized by the highest concentrations of the flavonoids apigenin (159.06 ± 4.43 $\mu\text{g/g}$ extract), eriodictyol (128.53 ± 1.84 $\mu\text{g/g}$ extract), naringenin (104.81 ± 2.67 $\mu\text{g/g}$ extract), and kaempferol (66.89 ± 0.79 $\mu\text{g/g}$ extract). In contrast, the methanol extract contained the highest levels of the phenolic acids ferulic acid (202.31 ± 2.07 $\mu\text{g/g}$ extract), chlorogenic acid (153.37 ± 0.73 $\mu\text{g/g}$ extract), and syringic acid (93.64 ± 2.06 $\mu\text{g/g}$ extract), together with higher concentrations of the flavonoids rutin (72.28 ± 1.95 $\mu\text{g/g}$ extract) and catechin (39.77 ± 0.36 $\mu\text{g/g}$ extract). The water extract generally contained lower concentrations of the identified phenolic compounds, although chlorogenic acid (111.40 ± 1.53 $\mu\text{g/g}$ extract), ferulic acid (87.42 ± 0.85 $\mu\text{g/g}$ extract), and syringic acid (59.87 ± 0.67 $\mu\text{g/g}$ extract) remained the predominant constituents.

3.3. Antioxidant activities

The antioxidant activities of the ethyl acetate, methanol, and water extracts of *G. unalii* were evaluated using six complementary in vitro assays. The $\text{IC}_{50}/\text{EC}_{50}$ values obtained for each assay are presented in Table 3, whereas the corresponding antioxidant activities expressed as Trolox or EDTA equivalents are summarized in Table 4. The integrated antioxidant performance of the extracts, calculated using the Relative Antioxidant Capacity Index (RACI), is illustrated in Fig. 2.

Table 3

EC₅₀ and IC₅₀ values (mg/mL) of the antioxidant and enzyme inhibitory activities of extracts from *G. unalii*.

Assay	Ethyl acetate	Methanol	Water	Standard
Phosphomolybdenum (EC ₅₀)	0.97 ± 0.01 ^a	1.01 ± 0.001 ^a	1.23 ± 0.01 ^a	0.39 ± 0.03 ^a
CUPRAC (EC ₅₀)	11.03 ± 0.96 ^a	5.80 ± 0.27 ^a	10.28 ± 0.64 ^a	0.23 ± 0.01 ^a
FRAP (EC ₅₀)	2.44 ± 0.06 ^a	1.64 ± 0.06 ^a	1.07 ± 0.01 ^a	0.16 ± 0.004 ^a
DPPH (IC ₅₀)	1.04 ± 0.003 ^a	1.50 ± 0.05 ^a	3.52 ± 0.07 ^a	0.17 ± 0.01 ^a
ABTS (IC ₅₀)	0.90 ± 0.01 ^a	0.62 ± 0.01 ^a	0.70 ± 0.004 ^a	0.048 ± 0.002 ^a
Metal chelating (IC ₅₀)	1.03 ± 0.03 ^a	1.04 ± 0.001 ^a	1.02 ± 0.01 ^a	0.015 ± 0.001 ^a
AChE (IC ₅₀)	1.13 ± 0.04 ^a	1.09 ± 0.02 ^a	1.05 ± 0.01 ^a	0.0034 ± 0.0003 ^a
BChE (IC ₅₀)	0.98 ± 0.02 ^a	0.99 ± 0.03 ^a	0.98 ± 0.02 ^a	0.0054 ± 0.0004 ^a
Tyrosinase (IC ₅₀)	1.46 ± 0.01 ^a	1.43 ± 0.01 ^a	4.80 ± 0.11 ^a	0.082 ± 0.001 ^a
α-Amylase (IC ₅₀)	3.56 ± 0.01 ^a	4.60 ± 0.01 ^a	26.69 ± 0.13 ^a	1.51 ± 0.02 ^a
α-Glucosidase (IC ₅₀)	0.95 ± 0.01 ^a	0.72 ± 0.01 ^a	na	0.97 ± 0.03 ^a
Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences among extracts according to one-way ANOVA followed by Tukey's HSD test (<i>p</i> < 0.05). IC ₅₀ represents the concentration required to achieve 50% inhibition. EC ₅₀ represents the concentration producing an absorbance of 0.500 in the phosphomolybdenum, CUPRAC, and FRAP assays. Standard compounds: Trolox (phosphomolybdenum, DPPH, ABTS, CUPRAC and FRAP), EDTA (metal chelating), galantamine (AChE and BChE), kojic acid (tyrosinase), and acarbose (α-amylase and α-glucosidase). na, no activity.				

Table 4

Antioxidant and enzyme inhibitory activities of extracts from *G. unalii* expressed as standard equivalent activities

Assay	Unit	Ethyl acetate	Methanol	Water
Phosphomolybdenum	mg TE/g extract	402.41 ± 2.40 ^a	384.80 ± 0.90 ^b	317.97 ± 2.40 ^c
DPPH	mg TE/g extract	20.92 ± 1.82 ^b	39.73 ± 1.82 ^a	22.41 ± 1.40 ^b
ABTS	mg TE/g extract	66.13 ± 1.67 ^c	98.37 ± 3.34 ^b	150.56 ± 0.89 ^a
CUPRAC	mg TE/g extract	160.38 ± 0.41 ^a	111.45 ± 3.94 ^b	47.55 ± 0.95 ^c
FRAP	mg TE/g extract	54.32 ± 0.49 ^c	78.76 ± 1.47 ^a	69.55 ± 0.37 ^b
Metal chelating	mg EDTAE/g extract	14.11 ± 0.40 ^a	13.96 ± 0.01 ^a	14.31 ± 0.11 ^a
AChE	mg GALAE/g extract	2.91 ± 0.09 [⊠]	3.02 ± 0.06 [⊠]	3.14 ± 0.03 [⊠]
BChE	mg GALAE/g extract	5.48 ± 0.10 [⊠]	5.47 ± 0.16 [⊠]	5.50 ± 0.12 [⊠]
Tyrosinase	mg KAE/g extract	55.45 ± 0.48 [⊠]	56.58 ± 0.19 [⊠]	16.89 ± 0.39 [⊠]
α-Amylase	mg ACE/g extract	421.57 ± 1.67 [⊠]	326.09 ± 0.80 [⊠]	56.24 ± 0.27 [⊠]
α-Glucosidase	mg ACE/g extract	589.60 ± 6.70 [⊠]	772.70 ± 9.07 [⊠]	na
Values are expressed as mean ± SD (n = 3). Different superscript letters within the same row indicate significant differences among extracts according to one-way ANOVA followed by Tukey's HSD test (<i>p</i> < 0.05). TE, Trolox equivalent; EDTAE, ethylenediaminetetraacetic acid equivalent; GALAE, galantamine equivalent; KAE, kojic acid equivalent; ACE, acarbose equivalent; na, no activity.				

The ethyl acetate extract exhibited the highest phosphomolybdenum activity ($EC_{50} = 0.97 \pm 0.01$ mg/mL; 402.41 ± 2.40 mg TE/g extract) and CUPRAC reducing capacity ($EC_{50} = 11.03 \pm 0.96$ mg/mL; 160.38 ± 0.41 mg TE/g extract). The methanol extract showed the strongest DPPH radical scavenging activity ($IC_{50} = 1.50 \pm 0.05$ mg/mL; 39.73 ± 1.82 mg TE/g extract) and FRAP reducing power ($EC_{50} = 1.64 \pm 0.06$ mg/mL; 78.76 ± 1.47 mg TE/g extract), whereas the water extract exhibited the highest ABTS radical scavenging activity ($IC_{50} = 0.70 \pm 0.004$ mg/mL; 150.56 ± 0.89 mg TE/g extract). No significant differences were observed among the extracts in the metal chelating assay (*p* > 0.05).

Integration of the results obtained from the six antioxidant assays using the RACI approach ranked the methanol extract as the most effective antioxidant sample (0.34 ± 0.03), followed by the ethyl acetate (0.02 ± 0.01) and water (-0.36 ± 0.06) extracts (Fig. 2).

3.4. Enzyme inhibitory activities

The inhibitory activities of the ethyl acetate, methanol, and water extracts of *Genista unalii* against acetylcholinesterase (AChE), butyrylcholinesterase (BChE), tyrosinase, α -amylase, and α -glucosidase were evaluated. The IC_{50} values obtained for the enzyme inhibition assays are presented in Table 3, whereas the corresponding inhibitory activities expressed as standard-equivalent values are summarized in Table 4.

No statistically significant differences were observed among the extracts in AChE or BChE inhibition ($p > 0.05$). The IC_{50} values ranged from 1.05 to 1.13 mg/mL for AChE and from 0.98 to 0.99 mg/mL for BChE. Similarly, the corresponding activities expressed as galantamine equivalents ranged from 2.91 to 3.14 mg GALAE/g extract for AChE and from 5.47 to 5.50 mg GALAE/g extract for BChE (Table 4).

The ethyl acetate and methanol extracts exhibited comparable tyrosinase inhibitory activities ($IC_{50} = 1.46 \pm 0.03$ and 1.43 ± 0.01 mg/mL, respectively), whereas the water extract showed significantly weaker activity ($IC_{50} = 4.81 \pm 0.11$ mg/mL; $p < 0.05$). The corresponding tyrosinase inhibitory activities expressed as kojic acid equivalents showed the same trend (Table 4).

The methanol extract exhibited the strongest α -glucosidase inhibitory activity ($IC_{50} = 0.72 \pm 0.03$ mg/mL), followed by the ethyl acetate extract (0.95 ± 0.03 mg/mL), whereas no α -glucosidase inhibitory activity was detected for the water extract. In contrast, the ethyl acetate extract exhibited the strongest α -amylase inhibitory activity ($IC_{50} = 1.51 \pm 0.04$ mg/mL), followed by the methanol (2.52 ± 0.08 mg/mL) and water (14.63 ± 0.15 mg/mL) extracts. The corresponding α -amylase and α -glucosidase inhibitory activities expressed as acarbose equivalents followed the same pattern (Table 4).

3.5. Pearson correlation analysis and hierarchical clustering

Pearson correlation analysis was performed to evaluate the relationships among phenolic composition, total phenolic content (TPC), total flavonoid content (TFC), antioxidant activities, and enzyme inhibitory activities. The correlation matrix together with the hierarchical clustering heatmap is presented in Fig. 3.

Strong positive correlations were observed between TPC and phosphomolybdenum activity ($r = 0.97$), CUPRAC ($r = 0.95$), FRAP ($r = 0.91$), and α -amylase inhibitory activity ($r = 0.96$). TFC showed strong positive correlations with DPPH radical scavenging activity ($r = 0.93$), metal chelating activity ($r = 0.94$), and α -glucosidase inhibitory activity ($r = 0.98$). Among the individual phenolic compounds, apigenin and eriodictyol exhibited strong positive correlations with DPPH radical scavenging activity and α -glucosidase inhibition, whereas chlorogenic acid, ferulic acid, and syringic acid were positively correlated with phosphomolybdenum, CUPRAC, FRAP, and α -amylase inhibitory activities.

Hierarchical clustering analysis grouped variables with similar correlation patterns into distinct clusters. TFC, apigenin, eriodictyol, DPPH radical scavenging activity, metal chelating activity, and α -glucosidase inhibition formed one major cluster, whereas TPC, chlorogenic acid, ferulic acid, syringic acid, phosphomolybdenum activity, CUPRAC, FRAP, and α -amylase inhibition were grouped within a second cluster (Fig. 3).

Discussion

The present study revealed that the extraction solvent strongly influenced the phytochemical composition and biological activities of *Genista unalii*. Although the ethyl acetate extract exhibited the highest total phenolic content, the methanol extract contained the highest total flavonoid content and displayed the most consistent antioxidant performance together with the strongest α -glucosidase inhibitory activity. These findings demonstrate that the biological activities of *G. unalii* are associated not only with the total abundance of phenolic constituents but also with their qualitative composition and solvent-dependent distribution. The significant correlations observed between specific phenolic compounds and individual biological activities further support the contribution of phenolic composition to the functional properties of the extracts.

The marked differences observed among the extracts are consistent with the polarity of the extraction solvents and the structural diversity of phenolic metabolites. Methanol and water produced higher extraction yields than ethyl acetate, indicating a greater recovery of polar constituents from the plant material, whereas ethyl acetate preferentially extracted moderately polar phenolics, resulting in the highest total phenolic content. In contrast, methanol proved more effective for recovering flavonoids, as reflected by its significantly higher total flavonoid content. LC–MS/MS analysis further demonstrated distinct solvent-dependent phenolic profiles, with the ethyl acetate extract being enriched in flavonoid aglycones, including apigenin, eriodictyol, naringenin, and kaempferol, whereas chlorogenic acid, ferulic acid, syringic acid, rutin, and catechin predominated in the methanol extract. Similar solvent-dependent phenolic distributions have been reported for several *Genista* species, including *G. ferox*, *G. lobelii*, and *G. januensis* subsp. *lydia* (Sebaihi-Harzoun et al. 2018; Pustahija et al. 2023; Boukaabache et al. 2024; Argon et al. 2025). Collectively, these findings indicate that solvent selection is a critical determinant of the phytochemical profile of *Genista* extracts and further support the value of phenolic composition as a complementary chemotaxonomic characteristic within the genus.

The antioxidant assays demonstrated that the biological activity of *G. unalii* extracts was influenced by both the concentration and composition of phenolic constituents. Although the ethyl acetate extract exhibited the highest total phenolic content, the methanol extract showed the highest overall antioxidant performance according to the RACI analysis. This observation indicates that antioxidant activity cannot be explained solely by the total amount of phenolics but is also determined by the relative abundance and synergistic interactions of individual compounds. Similar relationships have been reported for other *Genista* species, where extracts enriched in chlorogenic acid, ferulic acid, rutin, catechin, and related

flavonoids exhibited stronger reducing power and radical scavenging activities than extracts with higher total phenolic contents alone (Pustahija et al. 2023; Boukaabache et al. 2024; Argon et al. 2025).

Correlation analysis further supported this interpretation. Chlorogenic acid, ferulic acid, and syringic acid were strongly associated with phosphomolybdenum, CUPRAC, and FRAP activities, whereas apigenin and eriodictyol showed closer relationships with DPPH radical scavenging and α -glucosidase inhibition. These findings are consistent with the complementary mechanisms measured by different antioxidant assays and emphasize that no single assay fully represents the antioxidant potential of complex plant extracts. The integrated RACI approach therefore provided a more comprehensive evaluation by combining the results of multiple antioxidant methods into a single comparative index. This finding highlights the importance of integrating complementary antioxidant assays when evaluating the functional properties of medicinal plants.

The enzyme inhibitory activities of *G. unalii* extracts were also strongly influenced by extraction solvent, indicating that solvent-dependent differences in phenolic composition contributed to distinct inhibitory profiles. The methanol extract exhibited the strongest α -glucosidase inhibitory activity, whereas the ethyl acetate extract was more active against α -amylase and tyrosinase. These observations are consistent with the LC–MS/MS analysis, which revealed marked differences in the distribution of individual phenolic compounds among the extracts. Previous studies have demonstrated that flavonoids such as apigenin, kaempferol, eriodictyol, and rutin, together with phenolic acids including chlorogenic and ferulic acids, contribute to carbohydrate-hydrolyzing enzyme and tyrosinase inhibition through multiple and often complementary mechanisms (Boukaabache et al. 2024; Argon et al. 2025). The superior α -glucosidase inhibitory activity of the methanol extract is therefore likely associated with the combined contribution of these compounds rather than with a single dominant metabolite, highlighting the importance of phytochemical complexity in determining enzyme inhibitory activity.

The Pearson correlation analysis further supported these relationships by linking chlorogenic acid, ferulic acid, and syringic acid with reducing capacity assays, whereas apigenin and eriodictyol were more closely associated with DPPH radical scavenging and α -glucosidase inhibition. The hierarchical clustering analysis revealed two well-defined groups of phenolic constituents and biological activities, reflecting distinct phytochemical signatures among the solvent extracts. Collectively, these findings demonstrate that the biological properties of *G. unalii* are closely associated with qualitative differences in phenolic composition rather than with total phenolic content alone. Beyond explaining the observed biological activities, the solvent-dependent phenolic profiles identified in this study provide additional chemotaxonomic information for *G. unalii* and support the growing evidence that phenolic fingerprints represent valuable complementary characters for comparative studies within the genus *Genista*.

The present study provides the first comprehensive phytochemical and biological characterization of *G. unalii*, demonstrating that solvent-dependent differences in phenolic composition are closely associated with antioxidant and enzyme inhibitory activities. The combined application of LC–MS/MS profiling, multiple antioxidant assays, enzyme inhibition analyses, and multivariate statistical approaches enabled

a comprehensive evaluation of the biological potential of this newly described endemic species. Although the observed activities require further confirmation through bioactivity-guided isolation, mechanistic investigations, and in vivo studies, the present findings establish a valuable foundation for future pharmacological research. Moreover, the distinct phenolic fingerprints identified among the solvent extracts provide additional chemotaxonomic evidence that complements morphological characterization and contributes to a better understanding of phytochemical diversity within the genus *Genista* (Sebaihi-Harzoun et al. 2018; Boukaabache et al. 2024). These findings support the potential of *G. unalii* as a promising source of bioactive phenolic compounds and highlight the importance of integrating phytochemical and biological datasets in future comparative studies of endemic *Genista* taxa.

Conclusions

This study presents the first comprehensive phytochemical and biological characterization of *G. unalii*, a recently described endemic species from Türkiye. LC–MS/MS analysis revealed a solvent-dependent phenolic composition comprising twenty identified compounds, while ultrasonic-assisted extraction with different solvents produced distinct phytochemical profiles associated with different biological activities. Although the ethyl acetate extract contained the highest total phenolic content, the methanol extract exhibited the highest total flavonoid content together with the most consistent antioxidant performance and the strongest α -glucosidase inhibitory activity. Correlation and hierarchical clustering analyses further demonstrated that the biological activities of the extracts were more closely related to qualitative differences in phenolic composition than to total phenolic content alone, emphasizing the importance of individual phenolic constituents and their combined effects. Beyond demonstrating the pharmacological potential of *G. unalii* as a source of bioactive phenolics with antioxidant and enzyme inhibitory properties, this study provides new chemotaxonomic evidence for the genus *Genista* and establishes a valuable reference for future comparative phytochemical and biological investigations of endemic *Genista* species.

Declarations

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Declaration of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available within the article and its supplementary material.

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Figures

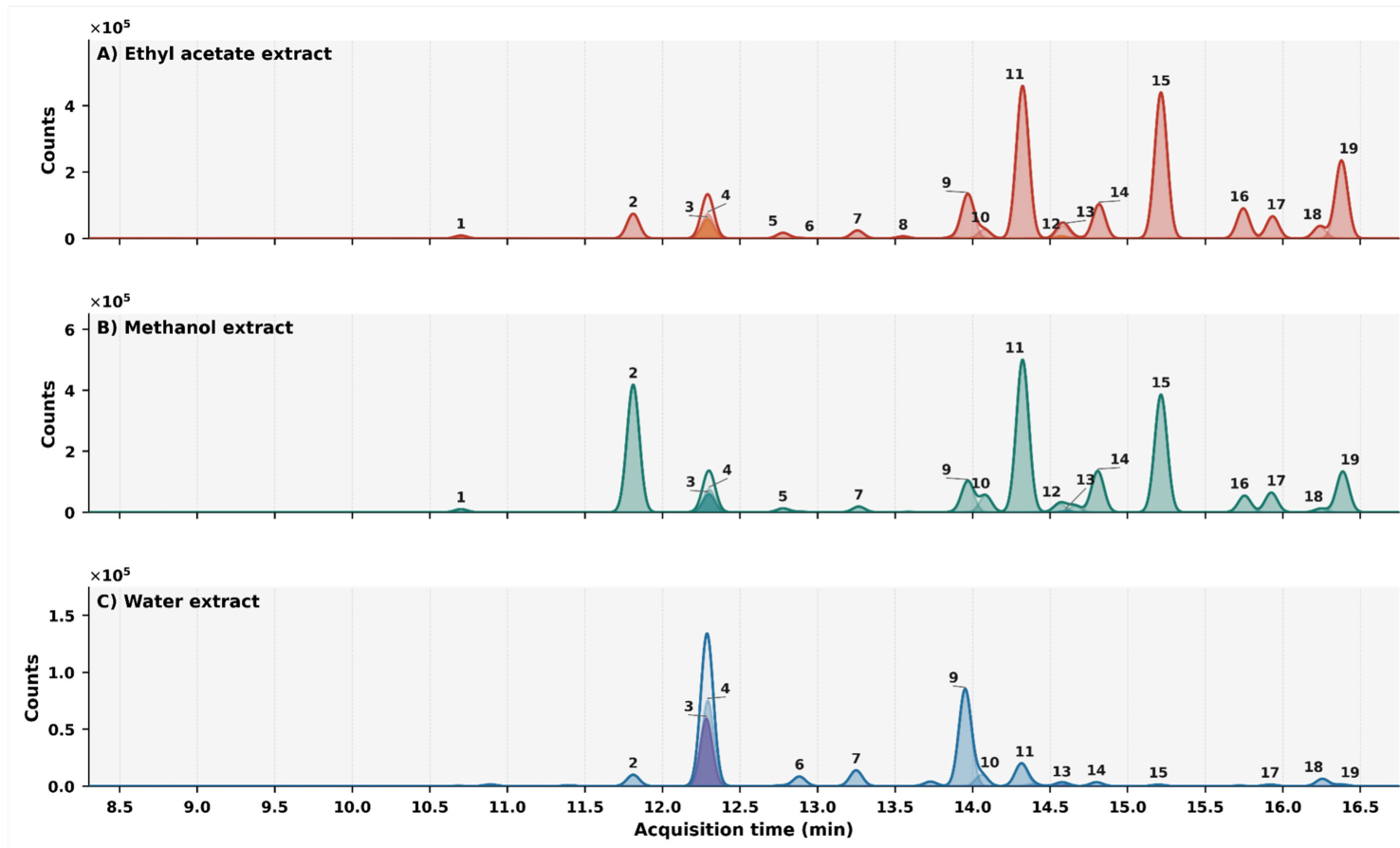


Figure 1

LC–MS/MS chromatograms of ultrasonic-assisted extracts obtained from the aerial parts of *G. unalii*: (A) ethyl acetate extract, (B) methanol extract, and (C) water extract. Peaks were identified according to their retention times and quantified by LC–MS/MS analysis. 1, Protocatechuic acid; 2, Chlorogenic acid; 3, 3-Hydroxybenzoic acid; 4, 4-Hydroxybenzoic acid; 5, Caffeic acid; 6, Syringic acid; 7, Vanillin; 8, Verbascoside; 9, *p*-Coumaric acid; 10, Ferulic acid; 11, Luteolin 7-glucoside; 12, Hyperoside; 13, Rosmarinic acid; 14, Apigenin 7-glucoside; 15, Eriodictyol; 16, Quercetin; 17, Luteolin; 18, Kaempferol; 19, Apigenin.

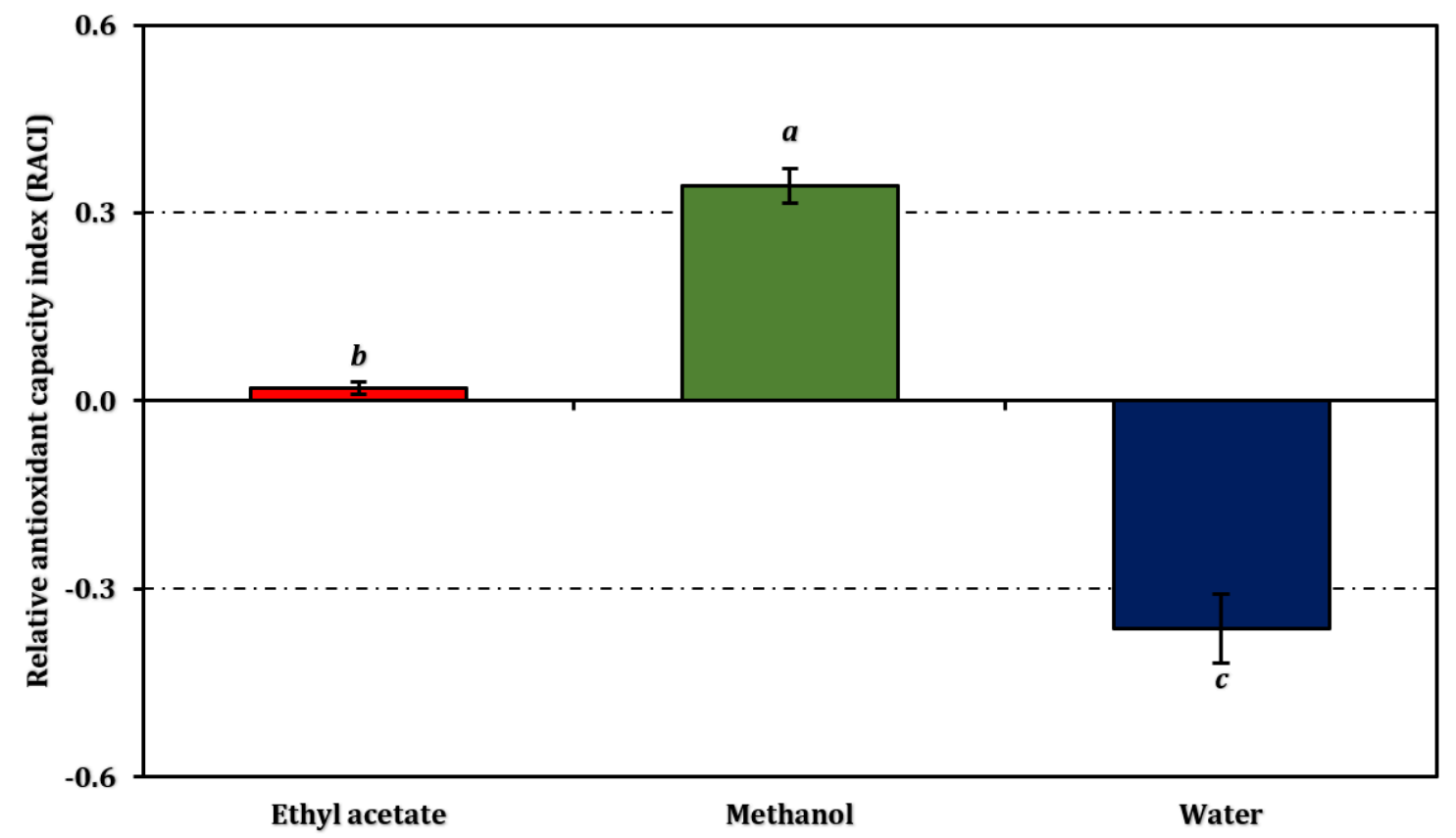


Figure 2

Relative antioxidant capacity index of ultrasonic-assisted extracts obtained from the aerial parts of *G. unalii*

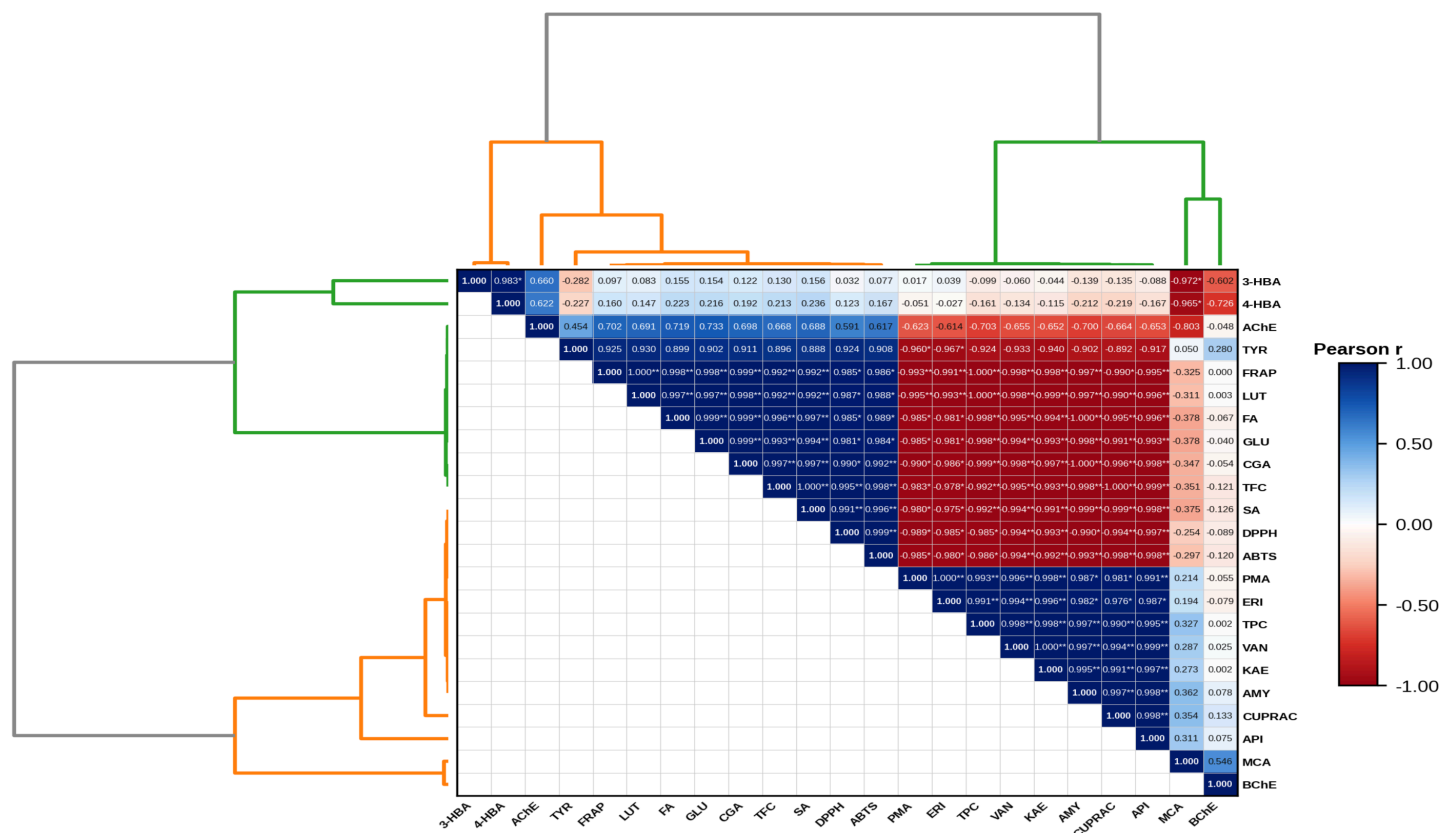


Figure 3

Hierarchically clustered Pearson correlation heatmap illustrating the relationships among antioxidant activities, enzyme inhibitory activities, total phenolic and flavonoid contents, and major phytochemicals identified in the extracts. Pearson correlation coefficients (r) are represented by a color scale ranging from -1 (red, negative correlation) to $+1$ (blue, positive correlation), with the numerical values displayed within each cell. Hierarchical clustering of both rows and columns was performed using the average linkage method based on Pearson correlation distances. $*p < 0.05$ and $**p < 0.01$ indicate statistically significant correlations. PMA, phosphomolybdenum assay; DPPH, 2,2-diphenyl-1-picrylhydrazyl radical scavenging assay; ABTS, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical cation scavenging assay; CUPRAC, cupric ion reducing antioxidant capacity; FRAP, ferric reducing antioxidant power; MCA, metal chelating activity; AChE, acetylcholinesterase inhibitory activity; BChE, butyrylcholinesterase inhibitory activity; TYR, tyrosinase inhibitory activity; AMY, α -amylase inhibitory activity; GLU, α -glucosidase inhibitory activity; TFC, total flavonoid content; TPC, total phenolic content; FA, ferulic acid; CGA, chlorogenic acid; ERI, eriodictyol; API, apigenin; 3-HBA, 3-hydroxybenzoic acid; 4-HBA, 4-hydroxybenzoic acid; LUT, luteolin; VAN, vanillin; SA, syringic acid; and KAE, kaempferol.

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