

Article

Cypriot *Cyclamen* spp.: Phytochemical Profiles, Evaluation of the Antioxidant, Antimicrobial Activities and α -Amylase, α -Glucosidase and Acetylcholinesterase Inhibitory Effects

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Abstract

Cyclamen cyprium Kotschy, an endemic species of Cyprus, remains largely unexplored in terms of its chemical content and biological activity, despite the well-documented effects of other *Cyclamen* spp. This study aimed to characterize the phytochemical composition and biological activities of leaf extracts from the endemic *C. cyprium* and to compare them with those of *Cyclamen persicum*, the most extensively investigated species within the genus, in order to evaluate interspecific variation in phytochemical composition and bioactivity. Plant extracts prepared with methanol, ethanol, and acetone were assessed for total phenolic and flavonoid content. Their antioxidant activity (DPPH, ABTS, and FRAP assays), metal chelating ability, antimicrobial activity, and possible inhibitory effect related to α -amylase, α -glucosidase and acetylcholinesterase were also evaluated. The results demonstrated that solvent type significantly influenced extraction efficiency, with acetone yielding the highest phenolic (127.88 mg gallic acid/g) and flavonoid (110.42 mg rutin/g) content, while methanol extracts exhibited the strongest antioxidant activity (IC₅₀ = 30.18 μ g/mL). Overall, *C. persicum* showed greater antioxidant capacity compared to *C. cyprium*, although both species demonstrated moderate antimicrobial activity (MIC = 0.31 mg/mL) and enzyme inhibitory activities (IC₅₀ values ranging from 14.07 to 39.64 μ g/mL). Present findings highlight the presence of bioactive compounds (such as gallic acid, caffeic acid, rutin, and quercetin) in *C. cyprium*, supporting their potential as a natural source of antioxidant and pharmacologically relevant agents. Studying both *C. cyprium* and *C. persicum* thus provides essential comparative context, revealing species-specific bioactivities and supporting the broader pharmaceutical relevance of the genus.

Keywords: *C. cyprium*; *C. persicum*; ABTS; DPPH; FRAP; amylase; glucosidase; antimicrobial activity



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1. Introduction

Cyclamen cyprium Kotschy is a perennial, geophytic flowering plant endemic to Cyprus, known as the island's national plant and protected within state forest lands due to its restricted biogeographical distribution and ecological significance. Despite the extensive ethnobotanical and phytochemical documentation available for several Mediterranean *Cyclamen* species, with the most notable being *C. persicum*, the bioactive profile of *C. cyprium* remains largely unexplored, leaving a clear knowledge gap in Cypriot pharmacognosy

and natural-product research [1]. *Cyclamen cyprium* is distinguished by its rounded to cordate, fleshy leaves with coarsely dentate margins and a characteristic deep purple to crimson-purple underside. The upper lamina may show subtle marbling and leaves typically emerge shortly after flowering. Its solitary, nodding autumn flowers bear long pedicels and a deeply five-lobed corolla, ranging from white to pale pink, with a prominent magenta M-shaped blotch at the base of each lobe (Figure 1a). The species occupies a broad ecological range across Cyprus—from Akamas peninsula to the Troodos and Pentadaktylos mountain ranges—thriving on shaded calcareous or igneous rocky slopes, forested hillsides, and stream banks between 0 and 1075 m. Much of its distribution lies within Natura 2000 protected areas, underscoring its ecological significance and conservation value [1,2].

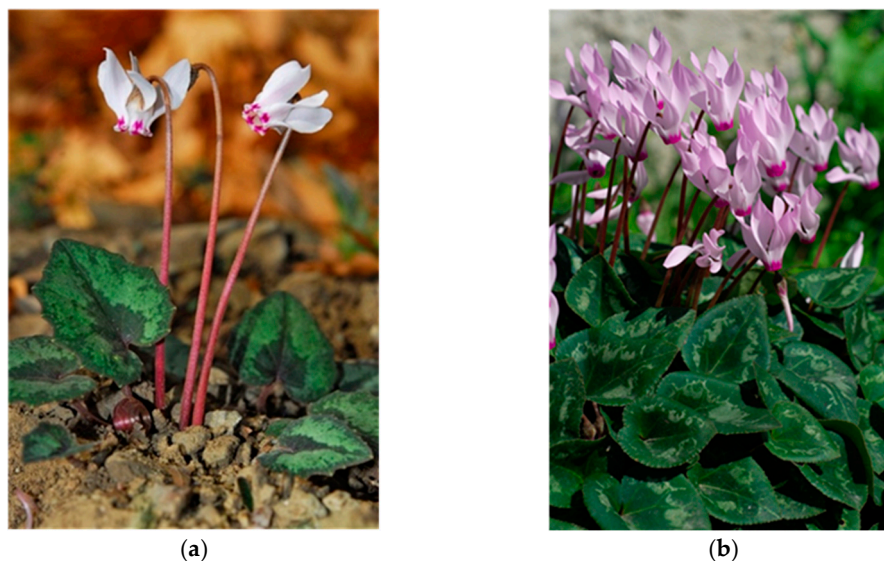


Figure 1. Morphological features of *C. cyprium* (a) and *C. persicum* (b) leaves, flowers, and corolla lobes (© Charalambos Christodoulou).

In contrast, *Cyclamen persicum* is a widely distributed eastern Mediterranean species, also common in Cyprus. It forms a large, flattened tuber from which arise long-petioled, broadly ovate to cordate leaves, often patterned in shades of green, gray, or silver, with a typically purplish underside. Its solitary flowers, produced from autumn to spring, are borne on long pedicels and characterized by deeply five-lobed, strongly reflexed corolla segments lacking basal auricles. Flower color ranges from white to deep carmine, usually with a darker magenta or purple zone around the corolla mouth (Figure 1b). *C. persicum* grows in maquis, garigue, open rocky habitats, limestone hillsides, woodland margins, and occasionally on old walls or in rock crevices from sea level to approximately 925 m. Its seasonal dormancy during the dry summer months reflects adaptation to Mediterranean climatic conditions [1]. These two species therefore represent contrasting ecological strategies within the same regional environment: one endemic and restricted, the other widespread and locally abundant. Their distinct morphological traits, habitat preferences and evolutionary histories provide a biologically meaningful framework for assessing whether endemic adaptation in *C. cyprium* is reflected in differentiated phytochemical signatures or biological activities of potential pharmacological and cosmetic interest.

The growing scientific interest in endemic flora is strongly linked to the urgent global need for new antioxidant and antimicrobial agents of plant origin, particularly as synthetic antioxidants raise safety concerns, whereas antimicrobial resistance continues to escalate, limiting the effectiveness of conventional therapies. Plants adapt to environmental stress through the production of secondary metabolites such as phenolic compounds, flavonoids, terpenoids, and triterpenoid saponins; molecular classes widely associated with free-

radical scavenging activity, reducing power, membrane-disrupting antimicrobial activity, and broad protective roles in chronic disease modulation [3,4].

Among *Cyclamen* spp., *C. persicum* remains the most extensively investigated, with studies consistently reporting high phenolic and flavonoid concentrations and corresponding antioxidant activity [5]. These works also identify key phenolic and flavonoid constituents—such as rutin, chlorogenic acid, quercetin, and kaempferol—supporting the species' established antioxidant and bioactive potential, alongside reports of mild antibacterial and broader biological activities [6]. Beyond the above constituents, triterpenoid saponins represent a major metabolite group in the genus; several structurally diverse saponins have been isolated from *C. mirabile* and *C. hederifolium*, highlighting their antimicrobial relevance and the rich saponin chemistry characteristic of *Cyclamen* taxa [6]. Additional studies on *C. coum* demonstrate notable antioxidant, enzyme-inhibitory, and antibacterial activities attributed primarily to saponins and phenolics [7,8]. Comparative analyses across species further show that pigment-associated phenolics, including anthocyanins and carotenoids, contribute substantially to antioxidant and antimicrobial properties within the genus [9], while investigations of *C. cilicium* and related taxa reinforce the broader pharmacological potential of *Cyclamen* spp. [10]. Collectively, these findings underscore the genus as a promising source of bioactive metabolites, supporting the rationale for investigating the phytochemical profile and antioxidant, antimicrobial, and anti-diabetic properties of Cypriot *Cyclamen* spp. in the present study. Among these species, *C. persicum* was selected for comparison because its phytochemical composition and biological activities have been more extensively characterized than those of any other member of the genus and co-occurs naturally in Cyprus with the endemic *C. cyprium*, allowing interspecific variation in phytochemical composition and biological activity to be evaluated within a common geographical context.

Natural products rich in phenolics and flavonoids have been frequently demonstrated to have cholinesterase-modulating properties [11], while several *Cyclamen* spp.—including *C. cilicium* and *C. coum*—have previously shown measurable cholinesterase inhibition [12,13]. Assessing AChE inhibition in *C. cyprium* and *C. persicum* therefore provides insight into whether these taxa share this pharmacologically relevant activity and helps clarify the potential of the genus as a source of neuroprotective metabolites.

Existing studies across the *Cyclamen* genus show that many species accumulate substantial concentrations of phenolics, flavonoids, and structurally diverse triterpene saponins, contributing to enzyme-inhibitory and occasionally cytotoxic activities [14]. Yet, despite this expanding biochemical evidence, the endemic *C. cyprium* has never been systematically characterized, leaving an important knowledge gap regarding how endemic adaptation may shape metabolite composition and biological activity.

Endemic plants frequently develop unique phytochemical profiles driven by localized ecological pressures, microclimate, etc., which may result in bioactivities distinct from those of widely distributed or cultivated species. Although *C. persicum* is well-studied globally, its Cypriot populations represent the common local taxon and therefore provide an essential regional reference for evaluating whether *C. cyprium* expresses differentiated biological activities within the same environmental context. To address this knowledge gap, the present study investigated the phytochemical composition and biological activities of the endemic *C. cyprium*. The inclusion of *C. persicum*, the most extensively investigated species within the genus, enabled the phytochemical composition and biological activities of *C. cyprium* to be evaluated in relation to a closely related species for which substantial phytochemical and pharmacological information is available. This dual comparison strengthens the ecological and biochemical relevance of the work by distinguishing traits linked to endemic adaptation from those shared across regional *Cyclamen* taxa. Accord-

ingly, the aim of this study was to characterize the phytochemical profile of *C. cyprium* leaf extracts and evaluate their antioxidant, antimicrobial, α -amylase, α -glucosidase and acetylcholinesterase inhibitory activities, while assessing interspecific variation through comparison with *C. persicum*.

2. Materials and Methods

2.1. Chemicals

The following solvents and chemicals were purchased from Sigma Aldrich, Hamburg, Germany and used in Sections 2.2–2.4: methanol, ethanol, acetone, acetonitrile, acetic acid, rutin, gallic acid, quercetin, caffeic acid, dimethyl sulfoxide (DMSO), Folin–Ciocalteu, aluminum chloride, 2,2'-diphenyl-1-picrylhydrazyl (DPPH), Trolox, 2,2'-azinobis (3-ethylbenzthiazoline-6-sulphonic acid (ABTS), potassium persulfate, 2,4,6-Tripyridyl-S-triazine (TPTZ), 40 mM HCl solution, anhydrous FeCl₃ (ferric chloride), acetate buffer pH 3.6, Iron(II) sulfate heptahydrate (FeSO₄·7H₂O), ferrozine and ethylenediaminetetraacetic acid (EDTA). For the antimicrobial assays (Section 2.5), the following media were used: Mueller–Hinton Broth II (MHB, Liofilchem, Roseto degli Abruzzi, Italy), Tryptic Soy Broth (TSB; Liofilchem, Roseto degli Abruzzi, Italy), ampicillin (Sigma-Aldrich, Hamburg, Germany), gentamicin (Molekula, Darlington, UK) and *p*-iodonitrotetrazolium chloride (INT) (Sigma-Aldrich, Gillingham, UK).

For the enzyme inhibition assays, the following chemicals were purchased from Sigma Aldrich, Hamburg, Germany: phosphate buffer (PB), starch, 3,5-dinitrosalicylic acid (DNSA), acarbose, α -glucosidase solution (G5003), *p*-nitrophenyl- α -D-glucopyranoside (*p*-NPG), phosphate-buffered saline (PBS), acetylcholinesterase (AChE) enzyme solution (G3389), 5,5-dithio-bis-(2-nitrobenzoic acid (DTNB), acetylthiocholine iodide (ATCI) and galanthamine. The solution of α -amylase (2 U/mL) was purchased from Sigma Aldrich, Hamburg, Germany.

2.2. Collection and Extraction of Plant Material

Plant leaves were collected from 20 randomly selected *C. persicum* and *C. cyprium* plants grown in the areas of Liopetri River and Alona, respectively. Coordinates of the central point of *C. persicum* surface are: 36S 582011 mE and 3869958 mN (altitude 5 m) and for *C. cyprium*: 36S 503676 mE and 3866241 mN (altitude 955 m). Both plant species were identified and distinguished from other *Cyclamen* spp. based on morphological characteristics, as previously described. Species identification was carried out by plant taxonomists. The species were confirmed using samples CYP 92 and 1966 for *C. cyprium* and samples CYP 2341 and 5674 for *C. persicum*, which are preserved in the Department of Forests Herbarium of the Ministry of Agriculture, Rural Development and Environment.

Leaf plant material (4 g, finely powdered) was subjected to extraction with 70 mL of each solvent (methanol, ethanol, and acetone). The plant–solvent mixtures were placed in an ultrasonic bath (Witeg Labortechnik GmbH, Hamburg, Germany) for 10 min to facilitate compound release. Following sonication, the suspensions were centrifuged at 4 °C (4000 rpm, 10 min) and the supernatants were passed through filter paper. Solvents were removed completely using a rotary evaporator (Stuart RE300, Keison, Chelmsford, UK) operating at 40 °C under reduced pressure (<1 mmHg). This extraction cycle was performed three times for each solvent, and the combined crude extracts were stored at 4 °C until analysis. Extraction yield for every sample was calculated using the formula: Extraction yield % = (Weight of dry extract/weight of raw material) $\times$ 100.

2.3. Chemical Analysis of the *Cyclamen* spp. Extracts

Bioactive compounds were analyzed using an RP-HPLC (Waters Alliance e2695, Manchester, UK) system equipped with a 2998 PDA detector. Data collection and processing were carried out with Empower-3 chromatography software. Separation was achieved on a C8 Column WAT035876 (Waters, UK) (60Å, 3.9 mm × 150 mm, 4 µm particle size). The mobile phase consisted of 0.5% acetic acid (solution A) and 0.5% acetic acid in acetonitrile (solution B). The flow rate was maintained at 0.7 mL/min, and the analysis was optimized in gradient elution mode as follows: 0–15 min 1–15% B, 25–45 min 25–55% B and 53–65 min 60–5%, while the column temperature was kept at approximately 25 °C. Prior to injection, samples were filtered through a 0.45 µm membrane filter, and a 20 µL injection volume was used. Detection of the target compounds was performed using a photodiode array (PDA) detector operating within a wavelength range of 210–400 nm. The chromatograms were examined at 270 nm with an LC gradient detector. Rutin, gallic acid, quercetin and caffeic acid were used as standards. The chromatographic peaks were assigned by matching their retention times and UV absorption profiles to those of authenticated reference standards. Quantification of each phenolic constituent was expressed as µg/mL of extract. To prepare the standard mixture, 5 mg of each compound were dissolved in 5 mL of 100% ethanol, followed by dilution to a final volume of 25 mL with distilled water. A 20 µL aliquot of the prepared standard mixture was injected into the RP-HPLC system and analyzed using the previously described reversed-phase RP-HPLC method. *Cyclamen* extracts weighing 100 mg were dissolved in 20 mL of 95% ethanol. Subsequently, 100 mL of distilled water was added to the solution. A 20 µL aliquot of the prepared extract was injected into the RP-HPLC system and analyzed using the previously described reversed-phase RP-HPLC method [5].

2.4. Measurements of Total Bioactive Compounds

2.4.1. Total Phenolic and Total Flavonoid Content

The total phenolic content (TPC) of *C. cyprium* and *C. persicum* extracts was determined using the Folin–Ciocalteu method [15]. A calibration curve for gallic acid was prepared using standards spanning 0.025–1.5 mg/mL. After a 30 min reaction period, absorbance was measured at 750 nm with a UV-1280 spectrophotometer (Shimadzu, Duisburg, Germany). The same analytical workflow was applied to the *Cyclamen* extracts, which were dissolved in DMSO to a final concentration of 5 mg/mL. Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric method [15]. Crude extracts were diluted in DMSO to a final concentration of 5 mg/mL, and absorbance was recorded at 415 nm using the extract solvent as the reference. Calibration curves were generated using rutin (0.02–0.2 mg/mL), quercetin (0.025–0.3 mg/mL), and catechin (0.02–0.5 mg/mL) standards. Results were expressed as milligrams of standard equivalents per gram of dried plant material.

2.4.2. DPPH Radical Scavenging Activity

The in vitro free radical scavenging capacity of the *Cyclamen* spp. extracts was evaluated using the DPPH assay, according to previously reported methodologies [16]. A 100 µL aliquot of the 0.5 mM DPPH working solution was combined with a range of extract concentrations. The mixtures were gently agitated and allowed to react for 30 min at room temperature in the dark. Absorbance was then recorded at 515 nm using a Sunrise microplate reader (TECAN Trading Ltd., Männedorf, Switzerland). Control and Trolox reference standards were also prepared and analyzed in parallel as the assay control.

Scavenging activity was quantified as the percentage of DPPH radicals neutralized and calculated using the following Equation (1):

$$\% \text{ scavenging activity} = \frac{(AB - AA)}{AB} \times 100 \quad (1)$$

where AB is the absorbance of the control sample, and AA is the absorbance of the sample.

The half-maximal inhibitory concentration (IC_{50}) corresponded to the extract concentration ($\mu\text{g/mL}$) necessary to scavenge 50% of the DPPH radicals.

2.4.3. ABTS Radical Scavenging Activity

ABTS cation scavenging activity was assessed as described in previous studies [17]. ABTS radical cations were generated by combining a 7 mM ABTS solution with 2.45 mM potassium persulfate, followed by overnight incubation in the dark. The $ABTS^+$ working solution was adjusted with 50% methanol until its absorbance at 734 nm reached approximately 0.70 ± 0.02 . To assess radical-scavenging activity, 100 μL of each extract (initial concentration 5 mg/mL) was combined with 1.9 mL of the diluted ABTS reagent. Trolox served as the reference antioxidant. All mixtures were kept in the dark for 30 min, after which absorbance was recorded at 734 nm using a Sunrise microplate reader (TECAN Trading Ltd., Männedorf, Switzerland). Inhibition of the $ABTS^+$ radical was calculated using Equation (1), and antioxidant capacity was expressed as IC_{50} , representing the extract concentration ($\mu\text{g/mL}$) required to achieve 50% radical reduction.

2.4.4. Ferric Reducing Antioxidant Activity (FRAP)

The FRAP reagent was prepared by mixing 20 mM TPTZ in 40 mM HCl with anhydrous FeCl_3 and 300 mM acetate buffer pH 3.6 at a ratio of 10:1:1 (acetate buffer: FeCl_3 :TPTZ). The working solution was pre-warmed at 37 °C for 30 min prior to use. Serial dilutions of each sample were prepared in 96-well microplates. An aliquot of 150 μL of the pre-warmed FRAP reagent was dispensed into each well, followed by incubation at 37 °C for 30 min. After incubation, absorbance was recorded at 593 nm using a Sunrise microplate reader (TECAN Trading Ltd., Männedorf, Switzerland). The FRAP activity was calculated from a FeSO_4 standard curve and expressed as $\mu\text{g Fe(II)}$ equivalents per milliliter of sample [18].

2.4.5. Metal Chelating Activity on Ferrous Ions

The metal chelating activity of the extracts was evaluated according to the ferrozine assay [19]. Briefly, 400 μL of sample solution at different concentrations (0.1–0.8 mg/mL) was mixed with 400 μL of 0.1 mM $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$. Moreover, 800 μL of 0.25 mM ferrozine was added to the mixture. The reaction mixture was incubated at room temperature for 10 min. The absorbance of the samples was then measured at 562 nm with the use of a spectrophotometer (V-630, Jasco International Co., Ltd., Cremella, Italy). EDTA was used as a reference compound. A control and a blank sample were also prepared and measured. All analyses were performed in triplicate, and the metal chelating effect was expressed as percentage of inhibition using Equation (1).

2.5. Evaluation of Antimicrobial Activity of *Cyclamen* spp. Extracts

The minimum inhibitory concentration (MIC) of *Cyclamen* extracts was determined using the broth microdilution method [20]. The extracts were prepared as a 10 mg/mL solution (100 μL final volume) in 10% DMSO and MHB media solution and were subjected to 2-fold serial dilutions. Bacterial strains *Escherichia coli* (NCTC 9001), *S. aureus* (NCTC 6571), *E. faecalis* (NCTC 775), and *S. enteritidis* (WDCM 00030) (Sigma-Aldrich, Hamburg, Germany) were cultured in TSB media and standardized to approximately 1×10^6 CFU/mL (100 μL final volume) using a spectrophotometer (V-630, Jasco International Co., Ltd.,

Cremella, Italy). Blank controls (10% DMSO, only extract), growth controls (only bacteria) and sterility controls (only media) were also included. Positive controls included bacteria supplemented with ampicillin (starting concentration 0.516 mg/mL) or gentamicin (starting concentration 0.064 mg/mL). After incubation of the samples for 18 h at 37 °C, 30 µL of INT (final 0.2 mg/mL) was added to each well. Following an additional incubation at 37 °C for 30 min, absorbance of all samples was measured at 492 nm using a microplate reader (Sunrise, Tecan Trading Ltd., Männedorf, Switzerland). The MIC was defined as the lowest concentration of the plant extract that successfully kept the INT dye colorless by completely arresting bacterial metabolic activity.

2.6. Evaluation of Enzyme Inhibitory Activities

2.6.1. α -Amylase Inhibition

The α -amylase inhibition assay was carried out according to the DNSA method [21]. Crude extracts of *Cyclamen* spp. were prepared in PB pH 6.9 at concentrations of 25–800 µg/mL. A reaction mixture consisting of 100 µL of α -amylase (2 U/mL) and 200 µL of extract was incubated at 37 °C for 20 min. Thereafter, 200 µL of the starch solution (1% in water (*w/v*)) was added to each tube and incubated for 30 min at 37 °C. The reaction was terminated by adding 1000 µL of DNSA reagent, followed by heating at 98 °C for 25 min in a water bath. After cooling to room temperature, absorbance was recorded at 540 nm using a UV-Visible spectrophotometer (V-630, Jasco International Co., Ltd., Cremella, Italy). A control corresponding to 100% α -amylase activity and blank samples were also prepared. Acarbose served as the positive control. The percentage inhibition of α -amylase was calculated using Equation (2):

$$\text{Inhibition (\%)} = [(Ac - Acb) - (As - Asb)/(Ac - Acb)] \times 100 \quad (2)$$

where Ac is the absorbance of control; Acb is the absorbance of control blank; As is the absorbance of sample; and Asb is the absorbance of sample blank. The IC₅₀ value was determined using the non-linear regression equation.

2.6.2. α -Glucosidase Inhibition

The α -glucosidase inhibitory activity of the extracts was evaluated using a standard method with minor modifications [22]. The assay was conducted in tubes containing 675 µL PBS (50 mM, pH 6.8), 100 µL α -glucosidase solution (10 U/mL, G5003), and 100 µL of extract or acarbose at varying concentrations (0.1–0.5 mg/mL). The mixture was pre-incubated at 37 °C for 20 min. Subsequently, 125 µL of 4 mM p-NPG in 50 mM PBS pH 6.8 was added as the substrate, and the plate was incubated further at 37 °C for 20 min. The samples' absorbance was measured at 405 nm. All experiments were performed in triplicate. The percentage inhibition of α -glucosidase activity was calculated using Equation (2), and the IC₅₀ value was determined using the non-linear regression equation.

2.6.3. Acetylcholinesterase Inhibition

The AChE assay was performed according to the ref. protocol [23]. Plant extract samples were diluted in 100 mM PBS pH 8.0. 20 µL of the sample was added to 140 µL PBS and 20 µL of AChE enzyme solution (1:10 dilution in PBS), and then it was incubated at room temperature for 10–15 min. Then 10 µL of 3 mM DTNB solution and 10 µL of 0.75 mM ATCI solution were added. After incubation for 15 min at room temperature, absorbance was measured at 405 nm. Appropriate blanks, negative controls and positive controls (Galanthamine) were included in all experiments. The percentage inhibition of α -glucosidase activity was calculated using Equation (2), and the IC₅₀ value was determined using the non-linear regression equation.

2.7. Statistical Analysis

Statistical evaluation was performed using GraphPad Prism (GraphPad Software 8.0, San Diego, CA, USA). Differences among groups were examined with one-way ANOVA followed by Tukey's multiple-comparison test. Results are presented as mean $\pm$ standard deviation (SD). All assays were performed in a minimum of three independent experiments, each including three biological replicates. A *p*-value below 0.05 was considered indicative of statistical significance.

3. Results and Discussion

The comparative analysis of *C. cyprium* and *C. persicum* revealed that both species share species-specific phytochemical characteristics and biological activities, providing the first comprehensive evaluation of the endemic *C. cyprium* within the context of an extensively studied member of the genus.

3.1. RP-HPLC Analysis of *Cyclamen* spp. Extracts

The reference-standard solution produced distinct and well-defined chromatographic peaks upon injection into the RP-HPLC system, providing clear spectral signatures for each compound. Photodiode array detection enabled simultaneous monitoring across the characteristic absorption maxima of the analytes, ensuring distinct identification of the phenolic and flavonoid constituents present in the *Cyclamen* extracts. Chromatograms of the standard mixture were recorded at 270 nm (Figure S1), resulting in the successful separation of all target compounds. The RP-HPLC chromatograms of *C. persicum* and *C. cyprium* extracts are presented in Figure 2. Chromatographic profiling of the extracts at 270 nm revealed strong absorbance signals for the major constituents. Compounds eluted over a broad window (5–65 min), reflecting the presence of metabolites spanning diverse polarity ranges. Alignment of retention times and UV spectral features with authenticated standards enabled the identification and quantification of multiple phenolic and flavonoid compounds (Table 1).

Table 1. RP-HPLC identification and quantification of *Cyclamen* spp. leaf extracts.

Bioactive Compounds	<i>C. persicum</i>		<i>C. cyprium</i>	
	Retention Time (RT)	Relative Abundance ($\mu\text{g/mL}$)	Retention Time (RT)	Relative Abundance ($\mu\text{g/mL}$)
Gallic acid	4.06	8.35	4.06	8.84
Caffeic acid	10.20	10.35	9.97	18.19
Rutin	13.79	26.96	15.00	83.49
Quercetin	44.33	200.88	44.35	219.76

RP-HPLC analysis of the leaf extracts of *C. persicum* and *C. cyprium* revealed the presence of at least four major phenolic constituents: gallic acid, caffeic acid, rutin, and quercetin. These compounds are widely distributed plant secondary metabolites and are known to contribute significantly to antioxidant, antimicrobial, as well as plant protection. The chromatographic profiles of both species were qualitatively similar, indicating a comparable phenolic composition. Gallic acid was detected at a retention time of approximately 4.06 min in both species, with concentrations of 8.35 $\mu\text{g/mL}$ in *C. persicum* and 8.84 $\mu\text{g/mL}$ in *C. cyprium* (Table 1). The relatively similar concentration of gallic acid suggests that gallic acid is a conserved metabolite within the genus. Gallic acid is a hydroxybenzoic acid commonly reported in medicinal plants and is recognized for its strong radical-scavenging activity and ability to protect plant tissues from oxidative stress [24].

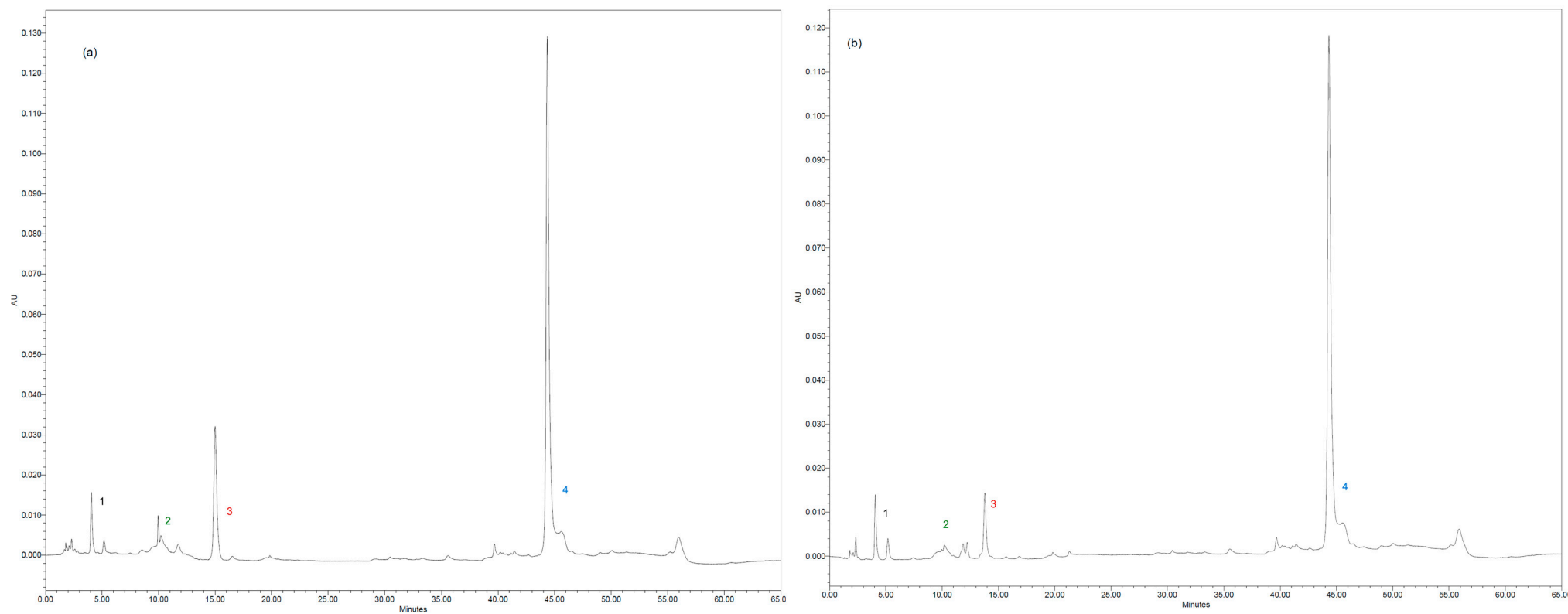


Figure 2. (a) RP-HPLC chromatograph of *C. cyprum*, (b) RP-HPLC chromatograph of *C. persicum*. Numbers 1–4 correspond to the following compounds: 1: Gallic acid, 2: Caffeic acid, 3: Rutin, 4: Quercetin.

Caffeic acid was identified at retention times of 10.20 min and 9.97 min in *C. persicum* and *C. cyprium*, respectively. However, its concentration was considerably higher in *C. cyprium* (18.19 µg/mL) than in *C. persicum* (10.35 µg/mL). Caffeic acid is an important hydroxycinnamic acid involved in the phenylpropanoid pathway and plays a key role in plant defense against pathogens, UV radiation, and environmental stress [25]. The elevated concentration in *C. cyprium* may reflect species-specific adaptations to the environmental conditions of Cyprus, where increased abiotic stress can stimulate phenolic biosynthesis.

Among the flavonoids detected, rutin showed a substantial difference between the two species. *C. cyprium* contained approximately three times more rutin (83.49 µg/mL) than *C. persicum* (26.96 µg/mL). Rutin (quercetin-3-rutinoside), one of the most abundant flavonoid glycosides in many plants, is well-known for its antioxidant, anti-inflammatory and antimicrobial properties [26]. In the present comparison, the noticeably higher rutin concentration measured in *C. cyprium* indicates a more substantial contribution to its antioxidant activity, suggesting that this compound may play a key role in enhancing the overall biological activity of *C. cyprium* relative to *C. persicum*.

Quercetin emerged as the predominant phenolic in both *Cyclamen* species, reaching concentrations of 200.88 µg/mL in *C. persicum* and 219.76 µg/mL in *C. cyprium*, making it the most abundant compound detected across all extracts. As one of the most biologically active plant flavonoids, quercetin is widely recognized for its strong free-radical-scavenging capacity, metal-chelating ability, and its role in modulating diverse cellular signaling pathways associated with stress responses [27]. Its pronounced dominance in both species therefore strongly suggests that quercetin is a major contributor to the antioxidant activity observed in the *Cyclamen* leaf extracts, likely underpinning a substantial portion of their overall bioactive potential.

Finally, the quantitative data indicate that *C. cyprium* accumulates higher amounts of caffeic acid, rutin, and quercetin than *C. persicum*, while gallic acid concentration remains comparable. This higher phenolic accumulation may be associated with genetic differences between the species and ecological adaptations to local environmental conditions. These findings also suggest that *C. cyprium* may possess stronger antioxidant and protective properties, attributable to its richer flavonoid composition, particularly the elevated accumulation of rutin and quercetin. This profile is consistent with previous phytochemical studies on *C. persicum*, which report high concentrations of phenolic acids and flavonoids contributing to the species' strong antioxidant potential. In particular, Al Rimawi et al. identified gallic acid, rutin, and quercetin as dominant constituents in *C. persicum* extracts, highlighting their role in the species' pronounced DPPH scavenging activity [5]. The concentrations measured here fall within the expected range for leaf tissues and further confirm quercetin as a key flavonoid in the species. Taken together, the consistency between our findings and previous studies reinforces the established phenolic richness of *C. persicum* and provides a solid comparative basis for interpreting the higher rutin (83.49 µg/mL) and quercetin (219.76 µg/mL) concentrations observed in the endemic *C. cyprium*.

3.2. Phenolic and Flavonoid Content

The extraction yield of the extracts was calculated according to the equation described in Section 2.2. All extracts exhibited relatively high recovery, indicating efficient solvent performance. Methanolic extraction produced yields of 26.2% for *C. persicum* and 26.7% for *C. cyprium*, while the ethanolic extracts yielded 21.5% and 22.3%, respectively. Acetone resulted in slightly lower but still satisfactory yields, with 18.6% for *C. persicum* and 19.5% for *C. cyprium*. The data presented in Figure 3 demonstrate that the extraction solvent plays a crucial role in determining the recovery of phenolic and flavonoid compounds from *Cyclamen* leaves. Among the tested solvents, acetone extracts consistently produced the

highest total phenolic content (TPC) and total flavonoid content (TFC) in both *C. persicum* and *C. cyprium*. This trend is clearly reflected in the quantitative results of the present study, where acetone yielded the highest TPC values for both species (122.91 mg/g in *C. persicum* and 127.88 mg/g in *C. cyprium*) and similarly produced the strongest flavonoid recovery (for rutin 98.53 mg/g and 110.42 mg/g, respectively). This finding is consistent with the intermediate polarity of acetone, which facilitates the extraction of a broad spectrum of phenolic compounds, including flavonoids and flavan-3-ols [27]. Similar solvent-dependent extraction patterns have been reported in other species of the genus. Studies on *C. coum* have demonstrated that different solvent systems significantly influence phenolic extraction efficiency and biological activity, with polar organic solvents generally yielding higher concentrations of phenolic constituents and stronger antioxidant activity. High-performance liquid chromatography analyses of *Cyclamen* species have revealed the presence of several phenolic compounds such as chlorogenic acid, caffeic acid, rutin, and kaempferol, compounds widely associated with antioxidant activity [5]. In the present study, acetone extraction yielded markedly higher TPC and TFC values in both species, with *C. cyprium* reaching 127.88 mg/g GAE and 110.42 mg/g RUE, compared with 122.91 mg/g GAE and 98.53 mg/g RUE in *C. persicum*. These values further support the superior efficiency of acetone for recovering phenolic constituents from *Cyclamen* leaves.

While solvent polarity strongly influenced extraction efficiency, the magnitude of flavonoid recovery also varied between species, indicating that both extraction conditions and inherent biochemical differences shape the phenolic profiles of *Cyclamen*. Although both species examined in the present study exhibited comparable total phenolic content in acetone extracts, distinct differences emerged in flavonoid composition and abundance. *C. cyprium* showed notably higher flavonoid content when expressed as catechin equivalents, particularly in the acetone extract (86.02 mg/g compared with 55.17 mg/g in *C. persicum*). This difference suggests species-specific variation in flavonoid biosynthesis and accumulation. These findings align with earlier comparative metabolomic studies reporting substantial variation in phenolic and flavonoid concentrations among *C. persicum*, *C. mirabile*, and *C. hederifolium*, highlighting the strong genetic and metabolic diversity within the genus [9]. The higher catechin-equivalent flavonoid content in *C. cyprium* (86.02 mg/g in acetone extract) compared with *C. persicum* (55.17 mg/g) reflects the same interspecific variability reported in previous metabolomic studies, where *Cyclamen* taxa differ substantially in their accumulation of flavonoids such as rutin, quercetin, and kaempferol. Furthermore, the present results indicate that *C. cyprium* not only accumulates higher total flavonoids but also displays elevated concentrations of specific flavonoids such as rutin (61.99 mg/g) compared with *C. persicum* (78.89 mg/g), reinforcing the species' comparatively richer flavonoid profile. This enhanced flavonoid content may contribute to the stronger antioxidant potential observed in *C. cyprium*, consistent with the literature describing flavonoids as key determinants of antioxidant activity in *Cyclamen* species.

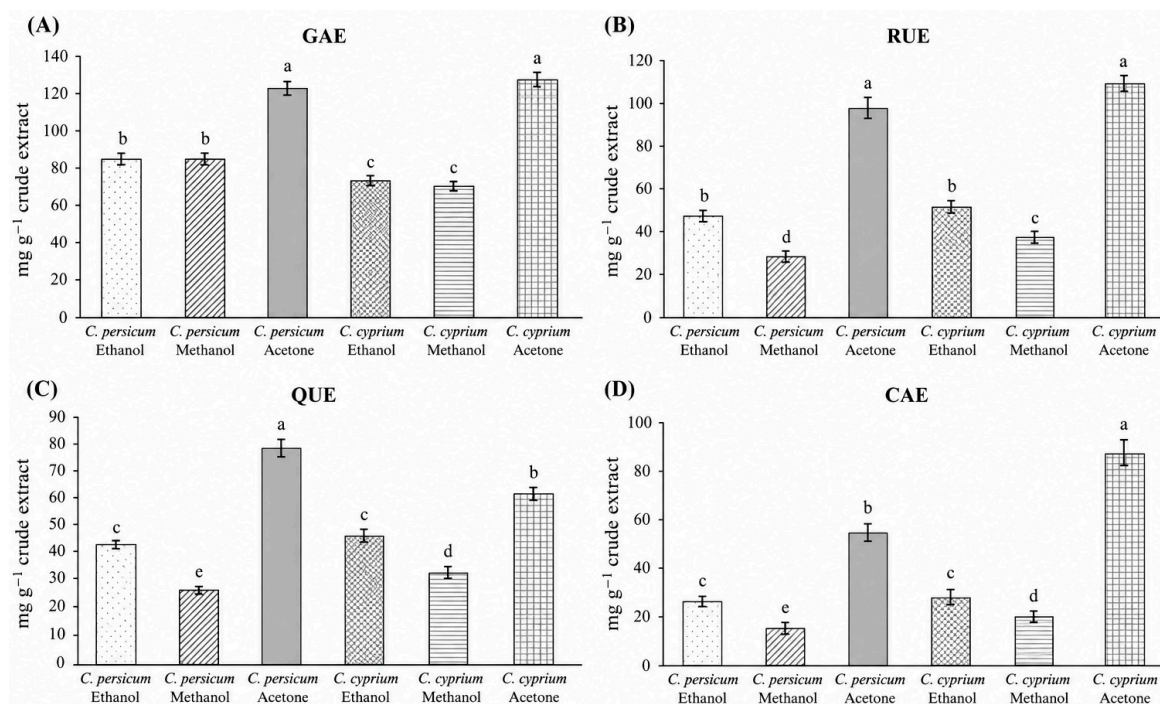


Figure 3. Total phenolic and total flavonoid content of *Cyclamen* spp. leaf extracts. (A) GAE: mg gallic acid equivalents per gram of crude extract, (B) RUE: mg rutin equivalents per gram of crude extract, (C) QUE: mg quercetin equivalents per gram of crude extract, (D) CAE: mg catechin equivalents per gram of crude extract. ^{a–e} Values having different letters differ significantly ($p < 0.05$).

3.3. Antioxidant Activity

Antioxidant activity, evaluated using DPPH and ABTS radical scavenging assays, showed that *C. persicum* possessed stronger antioxidant capacity than *C. cyprium*, as indicated by lower IC₅₀ values (Table 2). This trend was clearly reflected in the DPPH assay, where the methanolic extract of *C. persicum* reached an IC₅₀ of 34.58 µg/mL compared with 57.89 µg/mL in *C. cyprium*, and similarly in the ABTS assay (30.18 µg/mL vs. 48.14 µg/mL). Trolox showed a markedly lower IC₅₀ value, confirming its strong radical scavenging capacity compared to all plant extracts. Methanol extracts consistently exhibited the highest radical-scavenging activity in both species, likely due to the efficient extraction of hydrophilic phenolic compounds with strong antioxidant properties (Table 2). This observation aligns with the compound-specific profiles obtained in this study, where methanol extracts contained higher proportions of hydrophilic phenolics such as gallic acid and caffeic acid, both known for strong electron-donating and radical-neutralizing activity. Similar findings have been reported for *C. cilicium*, where methanolic extracts demonstrated significant activity in both DPPH and ABTS assays [28]. Despite acetone extracts containing the highest total phenolic and flavonoid contents, they did not always exhibit the strongest antioxidant activity, indicating that antioxidant potential depends on the structure, reactivity, and synergistic interactions of individual phenolic compounds rather than solely on their total concentration [29]. This discrepancy is consistent with the compound-specific profiles obtained in this study, where acetone extracts were richer in flavonoids such as rutin and quercetin—compounds contributing strongly to reducing power—while methanol extracts contained higher levels of simple phenolic acids that more effectively participate in radical-scavenging reactions. Differences between DPPH and ABTS results, particularly in *C. cyprium*, may reflect the distinct reaction mechanisms and antioxidant specificities of the assays, with ABTS detecting both hydrophilic and lipophilic antioxidants and DPPH being more responsive to lipophilic compounds [17]. These findings are consistent with reports of strong antioxidant activity in other *Cyclamen* spp., including *C. coum*, supporting the

genus as a promising source of natural antioxidants [30]. In the present study, the largest differences were observed in the DPPH assay, where *C. persicum* methanolic extract showed nearly twice the activity of *C. cyprium*, highlighting its more efficient electron-transfer-based antioxidant system. Together, these results highlight that solvent polarity and phenolic composition jointly shape the radical-scavenging behavior of *Cyclamen* extracts.

Table 2. Antioxidant activity of *Cyclamen* spp. leaf extracts determined by a combination of methods: DPPH and ABTS radical scavenging assays, FRAP total antioxidant activity and inhibition of metal-complex formation (metal chelating).

		DPPH	ABTS	FRAP	Metal Chelating (%) ²
		IC ₅₀ ¹ (µg /mL)	IC ₅₀ ¹ (µg /mL)	Equivalents FeSO ₄ (µg/mL) 100 µg/mL	100 µg/mL
<i>C. persicum</i>	Ethanol	43.56 ± 0.45 ^c	31.96 ± 1.23 ^b	25.44 ± 0.78 ^c	53.41 ± 1.45 ^c
	Methanol	34.58 ± 1.45 ^b	30.18 ± 2.34 ^b	73.65 ± 1.34 ^b	13.43 ± 1.04 ^d
	Acetone	49.66 ± 0.98 ^d	30.51 ± 1.09 ^b	15.86 ± 0.32 ^e	52.28 ± 1.36 ^c
<i>C. cyprium</i>	Ethanol	81.64 ± 1.67 ^f	87.80 ± 0.76 ^e	10.56 ± 0.97 ^f	56.18 ± 1.22 ^b
	Methanol	57.89 ± 1.57 ^e	48.14 ± 1.84 ^c	19.53 ± 1.21 ^d	11.38 ± 1.15 ^d
	Acetone	78.00 ± 0.72 ^f	77.69 ± 1.58 ^d	11.77 ± 0.65 ^f	56.27 ± 2.30 ^b
Positive control ³		5.02 ± 0.98 ^a	3.75 ± 1.02 ^a	82.67 ± 1.56 ^a	93.56 ± 1.23 ^a

IC₅₀ ¹: value for 50% inhibition of radical; % Metal chelating ²: Percentage of inhibition of metal-complex formation; Positive control ³: Trolox for DPPH, ABTS, FRAP and EDTA for metal chelating; SD: Standard deviation. ^{a-f} Values having different letters differ significantly ($p < 0.05$).

Beyond radical-scavenging assays, additional antioxidant mechanisms were evaluated using FRAP and metal-chelating assays to capture the broader spectrum of antioxidant behavior in both species. Extraction solvents also strongly influenced FRAP (expressed as FeSO₄ equivalents, µg/mL) and metal-chelating activity (expressed as percentage of inhibition of metal-complex formation of ferrozine) at a concentration of 100 µg/mL. *C. persicum*, particularly its methanolic extract, exhibited the highest FRAP values, suggesting a greater abundance of electron-donating phenolic compounds (Table 2). For example, the methanolic extract of *C. persicum* reached 73.65 µg/mL FeSO₄ equivalents, substantially higher than the corresponding *C. cyprium* extract (19.53 µg/mL), reflecting the higher abundance of electron-donating phenolics quantified in this species. This observation agrees with previous studies identifying rutin, chlorogenic acid, quercetin, and kaempferol as major antioxidant constituents of *C. persicum* [5,9]. In contrast, *C. cyprium* showed lower FRAP values across all solvents, likely reflecting differences in the composition of antioxidant compounds. Although limited literature is available specifically for *C. cyprium*, differences in FRAP activity may largely be attributed to differences in secondary metabolite profiles among *Cyclamen* spp. [9]. Therefore, the lower reducing power of *C. cyprium* observed in this study is likely species-dependent and reflects inherent phytochemical differences.

Regarding metal chelating activity, both species exhibited moderate to high inhibition of metal-complex formation values in ethanol and acetone extracts, while methanol extracts showed consistently low activity. Similar assay-dependent variations have been reported in *C. cilicium*, where antioxidant evaluation using multiple assays (including FRAP and metal chelation) showed that different extracts exhibited distinct activity profiles depending on their chemical composition. Although methanolic extracts showed strong reducing power, other extracts demonstrated better performance in complementary assays, highlighting the multi-mechanistic nature of antioxidant activity [31]. The inverse relationship between FRAP and metal-chelating activity observed in this study suggests that different

classes of phytochemicals contribute to these mechanisms: methanol-extracted phenolics primarily support electron-transfer-based antioxidant activity, whereas metal chelation may be associated with other constituents such as saponins, organic acids, or peptides. This pattern is consistent with the phenolic profiles of the extracts, as flavonoids such as rutin and quercetin—abundant in acetone extracts—are known to contribute strongly to metal chelation, whereas simple phenolic acids such as gallic and caffeic acid, more efficiently extracted with methanol, are potent electron donors that enhance FRAP activity. Overall, the combined results from radical-scavenging, reducing-power, and metal-chelating assays demonstrate that antioxidant activity in *Cyclamen* is shaped by both solvent polarity and species-specific phytochemical composition, with *C. persicum* showing consistently stronger activity across most mechanisms.

3.4. Antimicrobial Activity of *Cyclamen* spp. Extracts

The antibacterial activity of *C. persicum* and *C. cyprium* leaf extracts was evaluated against two Gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*) and two Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*). The MIC values are presented in Table 3 as mg/mL (mean $\pm$ SD). Ampicillin (Amp) and Gentamicin (Gen) were used as positive controls. Ethanolic extracts generally exhibited the strongest antibacterial effects, particularly in *C. persicum*, which showed the highest activity against *E. coli*. Specifically, the ethanolic extract of *C. persicum* achieved a MIC of 0.63 mg/mL against *E. coli*, compared with 1.25 mg/mL for *C. cyprium*, indicating a clear difference in susceptibility. Gram-positive bacteria were more susceptible to the extracts than Gram-negative bacteria. The greatest antibacterial activity was observed against *E. faecalis*, followed by *S. aureus*, with ethanolic extracts of both species producing the lowest MIC values. MIC values against *E. faecalis* reached as low as 0.16 mg/mL in both species, highlighting strong activity toward this Gram-positive pathogen. Activity against *S. enteritidis* was similar between the two species, although the acetone extract of *C. persicum* was less effective. Overall, *C. cyprium* extracts displayed slightly stronger antibacterial activity compared to that of *C. persicum*. This increased activity in *C. cyprium* may reflect its higher concentrations of key antimicrobial phenolics, such as rutin and quercetin, compared with *C. persicum*, as demonstrated in the present study. These compounds are widely associated with membrane-disruptive and enzyme-inhibitory antibacterial mechanisms, suggesting that phytochemical composition plays a central role in the observed interspecific differences [31]. Nevertheless, all plant extracts were considerably less potent than the reference antibiotics ampicillin and gentamicin, which exhibited substantially lower MIC values.

Table 3. Minimum inhibitory concentration (MIC) of the *Cyclamen* spp. against *E. coli*, *S. enteritidis*, *S. aureus* and *E. faecalis* bacteria.

MIC ¹ (mg/mL)		<i>E. coli</i>	<i>S. aureus</i>	<i>E. faecalis</i>	<i>S. enteritidis</i>
<i>C. persicum</i>	Ethanol	0.63 $\pm$ 0.04 ^c	0.31 $\pm$ 0.02 ^c	0.16 $\pm$ 0.05 ^b	0.63 $\pm$ 0.08 ^c
	Methanol	1.25 $\pm$ 0.08 ^d	0.63 $\pm$ 0.02 ^d	0.31 $\pm$ 0.05 ^c	0.63 $\pm$ 0.06 ^c
	Acetone	1.25 $\pm$ 0.84 ^d	0.63 $\pm$ 4.93 ^d	0.63 $\pm$ 0.84 ^c	1.25 $\pm$ 4.93 ^d
<i>C. cyprium</i>	Ethanol	1.25 $\pm$ 0.03 ^d	0.31 $\pm$ 0.08 ^c	0.16 $\pm$ 0.07 ^b	0.63 $\pm$ 0.04 ^c
	Methanol	1.25 $\pm$ 0.09 ^d	0.63 $\pm$ 0.04 ^d	0.31 $\pm$ 0.08 ^c	1.25 $\pm$ 0.04 ^d
	Acetone	1.25 $\pm$ 0.09 ^d	0.63 $\pm$ 0.08 ^d	0.31 $\pm$ 0.03 ^c	0.63 $\pm$ 0.05 ^c
Positive control	Amp ²	0.16 $\pm$ 0.01 ^b	0.05 $\pm$ 0.02 ^b	0.08 $\pm$ 0.01 ^a	0.14 $\pm$ 0.02 ^b
	Gen ³	0.04 $\pm$ 0.01 ^a	0.02 $\pm$ 0.01 ^a	0.14 $\pm$ 0.03 ^b	0.02 $\pm$ 0.01 ^a

MIC (mg/mL)¹: minimum inhibitory concentration; Amp²: Ampicillin positive control; Gen³: Gentamycin positive control. ^{a–d} Values having different letters in the same column differ significantly ($p < 0.05$).

The antibacterial assays revealed that both *C. persicum* and *C. cyprium* leaf extracts possess measurable antimicrobial activity against several bacterial strains. A clear pattern was observed in which Gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) were more susceptible to the plant extracts than Gram-negative bacteria (*Escherichia coli* and *Salmonella enteritidis*). This difference is commonly reported in studies of plant-derived antimicrobial compounds and can be explained by structural differences in bacterial cell envelopes. Gram-negative bacteria possess an outer membrane containing lipopolysaccharides, which acts as an additional permeability barrier and reduces the penetration of antimicrobial compounds [32]. The particularly strong activity observed against *E. faecalis* (MIC values as low as 0.15–0.16 mg/mL) highlights the potential of *Cyclamen* extracts as antibacterial agents against Gram-positive pathogens. Among the tested solvents, ethanol extracts demonstrated the lowest MIC values for both plant species, indicating the highest antibacterial efficacy. This solvent-dependent pattern aligns with the phytochemical profiles obtained in this study, as ethanol extracted higher levels of phenolics and flavonoids associated with antimicrobial activity, including rutin and quercetin. The stronger performance of ethanolic extracts in *C. persicum* compared with *C. cyprium* further supports the idea that the latter contains lower quantities of ethanol-soluble antimicrobial phenolics, consistent with its overall lower phenolic and flavonoid content. This suggests that ethanol may be particularly effective in extracting antimicrobial compounds from *Cyclamen* leaves. Phenolic compounds, flavonoids, and saponins, which have been previously reported in *Cyclamen* species, are known to possess antimicrobial properties through mechanisms such as membrane disruption, enzymatic inhibitory mechanisms, and interference with microbial metabolism [32].

Comparable antimicrobial properties have been reported in other *Cyclamen* species. Extracts of *C. coum* have demonstrated antibacterial activity against several bacterial pathogens, supporting the presence of biologically active antimicrobial compounds within the genus [7]. However, unlike the more widely distributed Mediterranean species such as *C. coum* and *C. cilicium*, *C. cyprium* is an endemic taxon restricted to Cyprus, and its narrower ecological range may contribute to distinct secondary-metabolite profiles. Endemic species often exhibit reduced chemical diversity compared with broadly distributed taxa, which may partly explain the lower antibacterial potency observed in *C. cyprium*. Although the MIC values were higher than those of conventional antibiotics such as ampicillin and gentamicin, their activity at relatively low mg/mL concentrations remains noteworthy. It is also important to note that crude extracts contain complex mixtures of metabolites, and the purification of active constituents—such as specific phenolic acids or saponins—typically results in significantly enhanced antimicrobial potency. Overall, the combined results indicate that antibacterial activity in *Cyclamen* is shaped by both solvent extraction efficiency and species-specific phytochemical composition, with *C. persicum* slightly outperforming the endemic *C. cyprium* across most bacterial strains.

3.5. Enzyme Inhibitory Activities

α -Amylase, α -glucosidase, and acetylcholinesterase were selected because they are clinically relevant therapeutic targets associated with type 2 diabetes and Alzheimer's disease, making them widely used biomarkers for evaluating the pharmacological potential of medicinal plant extracts. The enzymatic inhibitory activities of *C. persicum* and *C. cyprium* extracts varied according to both species and extraction solvent (Table 4). In *C. persicum*, the methanolic extract exhibited the highest α -glucosidase inhibitory activity (IC_{50} = 14.07 μ g/mL, the lowest value among all extracts), whereas the ethanolic extract showed the strongest acetylcholinesterase (AChE) inhibition (IC_{50} = 29.62 μ g/mL). In contrast, acetone showed moderate activity, with α -amylase inhibition (IC_{50} = 29.47 μ g/mL)

comparable to ethanol and higher α -glucosidase inhibition ($IC_{50} = 30.87 \mu\text{g/mL}$) than methanol. For *C. cypricum*, α -amylase inhibition was relatively similar among extracts ($IC_{50} = 22\text{--}23 \mu\text{g/mL}$), while the ethanolic extract showed the highest α -glucosidase inhibition ($IC_{50} = 20.18 \mu\text{g/mL}$), and the acetone extract demonstrated the strongest AChE inhibitory activity ($IC_{50} = 29.41 \mu\text{g/mL}$). No AChE inhibition was detected in the methanolic extract of *C. cypricum*. All extracts were considerably less active than the positive controls, indicating moderate inhibitory effects.

Table 4. α -amylase, α -glucosidase and acetylcholinesterase (AChE) inhibition activity and IC_{50} ($\mu\text{g/mL}$) of *Cyclamen* spp. leave extracts.

		α -Amylase	α -Glucosidase	AChE
		IC_{50}^1 ($\mu\text{g/mL}$)	IC_{50}^1 ($\mu\text{g/mL}$)	IC_{50}^1 ($\mu\text{g/mL}$)
<i>C. persicum</i>	Ethanol	28.52 ± 1.23^c	27.39 ± 1.78^c	29.62 ± 0.54^b
	Methanol	30.48 ± 0.87^b	14.07 ± 1.24^a	35.70 ± 1.20^d
	Acetone	29.47 ± 0.45^e	30.87 ± 0.76^d	33.45 ± 0.59^c
<i>C. cypricum</i>	Ethanol	23.48 ± 1.45^d	20.18 ± 1.29^b	39.64 ± 1.89^e
	Methanol	22.82 ± 1.02^d	30.92 ± 1.37^d	NA
	Acetone	23.17 ± 0.98^d	NA	29.41 ± 0.32^b
Positive Control ²	-	38.20 ± 1.56^a	35.20 ± 0.90^e	10.25 ± 0.56^a

IC_{50}^1 : value for 50% inhibition of enzyme; Positive control²: acarbose for α -amylase and α -glucosidase, galanthamine for AChE; NA: Not applicable; ^{a-e} Values having different letters differ significantly ($p < 0.05$).

Evaluation of the α -amylase data showed that *C. cypricum* ($IC_{50} \approx 22\text{--}23 \mu\text{g/mL}$) exhibits slightly stronger inhibition than *C. persicum* ($28\text{--}30 \mu\text{g/mL}$), which aligns with its higher caffeic acid content, a phenolic acid reported to inhibit both α -amylase and α -glucosidase in a dose-dependent manner [33]. In contrast, α -glucosidase inhibition is clearly dominated by *C. persicum*, whose methanol extract shows a markedly lower IC_{50} ($14.07 \mu\text{g/mL}$) compared with all *C. cypricum* extracts ($20\text{--}31 \mu\text{g/mL}$), suggesting that rutin and quercetin in *C. cypricum* may not be as efficiently extracted or may act less strongly on α -glucosidase. AChE inhibition follows a different pattern: *C. cypricum* acetone extract ($IC_{50} = 29.41 \mu\text{g/mL}$) performs comparably to *C. persicum* ethanol extract, consistent with the higher quercetin content in *C. cypricum*, as quercetin is a known cholinesterase inhibitor [34].

The observed solvent-dependent variations are likely associated with differences in phytochemical extraction efficiency. Species of the genus *Cyclamen* are known to contain phenolics, flavonoids, and triterpenoid saponins, compounds frequently associated with enzyme inhibitory activity [10]. The superior activity of the methanolic extract of *C. persicum* may reflect the greater ability of methanol to extract polar bioactive constituents, particularly phenolic compounds, which have been widely reported as inhibitors of carbohydrate-hydrolyzing enzymes [10]. This interpretation is supported by the present phytochemical data, where methanol extracts contained higher levels of gallic acid and caffeic acid—phenolics strongly linked to α -amylase and α -glucosidase inhibition. Similar findings were reported for *C. coum*, where methanolic extracts showed stronger enzyme inhibitory effects than extracts obtained with other solvents [7]. Despite the higher concentrations of caffeic acid, rutin and quercetin detected in *C. cypricum* by RP-HPLC, its enzyme inhibitory activity was generally lower than that of *C. persicum*. This discrepancy suggests that enzyme inhibition depends not only on the abundance of individual phenolics but also on their extractability, synergistic interactions and the presence of additional unidentified metabolites.

The moderate α -amylase and AChE inhibitory activities observed in both species are consistent with previous reports on other *Cyclamen* taxa. Specifically, *C. cilicium* extracts have been shown to inhibit cholinesterases, α -amylase, and α -glucosidase, although the potency depended on the extraction solvent and plant material used [28]. The comparatively lower AChE inhibition observed in the present study further supports the view that cholinesterase inhibition is generally less pronounced in *Cyclamen* species than carbohydrate-hydrolyzing enzyme inhibition. The absence of AChE inhibition in the methanolic extract of *C. cyprium* suggests that quercetin alone is insufficient to explain cholinesterase inhibition and that synergistic interactions with other metabolites or solvent-dependent extraction of active constituents are likely involved. Several *Cyclamen* spp., including *C. cilicium* and *C. coum*, have previously demonstrated cholinesterase-modulating activity, indicating that members of the genus may contain metabolites with neuroprotective potential [7,8,30]. Integrating AChE assays into this study therefore provides important context for assessing whether *C. cyprium* and *C. persicum* share this pharmacologically relevant property and helps clarify the broader therapeutic relevance of *Cyclamen* species.

Although *C. cyprium* contained higher concentrations of several phenolic compounds identified by RP-HPLC, *C. persicum* generally displayed stronger enzyme inhibitory activity. These findings indicate that biological activity is governed not only by the abundance of individual metabolites but also by their chemical interactions, bioavailability and extraction efficiency. The lower activity of all crude extracts relative to the standard inhibitor molecules suggests that further fractionation and isolation of active constituents are required to identify compounds with stronger pharmacological potential. Endemic species such as *C. cyprium* may also exhibit unique metabolite interactions that influence enzyme inhibition differently from more widely distributed taxa. Nevertheless, the results highlight *Cyclamen*, particularly *C. persicum*, as a promising source of natural enzyme inhibitors with potential applications in the management of diabetes and neurodegenerative disorders. The inclusion of *C. persicum* enabled the biological significance of the findings obtained for *C. cyprium* to be interpreted relative to a well-characterized *Cyclamen* species, demonstrating which phytochemical traits are conserved within the genus and which appear to be species-specific.

4. Conclusions

This study provides the first comprehensive assessment of the phytochemical profile and the biological activities of *C. cyprium*, an endemic species of Cyprus, alongside a comparative evaluation with the extensively studied *C. persicum*. Both species were shown to contain substantial concentrations of phenolic compounds, flavonoids, and other secondary metabolites, and demonstrated measurable antioxidant, antibacterial and enzyme inhibitory activities.

Solvent choice significantly influences extraction efficiency and biological performance. Acetone extracts yielded the highest phenolic and flavonoid contents, methanol extracts exhibited the strongest antioxidant activity, and finally, ethanol extracts showed the most pronounced antibacterial effects, particularly against Gram-positive bacteria. These findings highlight the role of solvent polarity in selectively extracting different classes of bioactive compounds.

Although *C. cyprium* displayed slightly lower antioxidant activity overall, its metal chelating activity was comparable to that of *C. persicum*, indicating the presence of constituents capable of mitigating metal-driven oxidative reactions. The consistent bioactivity observed across assays underscores *C. cyprium* as a promising, yet underexplored, source of natural phytochemicals.

Future work should focus on isolating and characterizing additional individual bioactive compounds, investigating mechanisms of action and evaluating potential synergistic

effects among phytochemicals. In vivo studies will also be essential to determine the pharmacological relevance of these findings. Overall, the present study expands current knowledge of *C. cyprium* and reinforces the value of endemic Mediterranean flora as rich reservoirs of biologically active natural products.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/appliedchem6030054/s1>, Figure S1: RP-HPLC chromatograph of standards.

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