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ORIGINAL ARTICLE

Comprehensive phytochemical profiling and cell-free in vitro bioactivity assessment of the endemic *Campanula kirikkaleensis* Dönmez & Güner

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Abstract: This study presents the first comprehensive evaluation of the phytochemical profile and cell-free antioxidant and enzyme inhibitory activities of the endemic *Campanula kirikkaleensis* Dönmez & Güner. n-Hexane, chloroform, and 70% methanol extracts from aerial parts and roots were investigated for their phytochemical composition and bioactivities. GC-FID/MS analyses revealed phytol (14.2%) as the main volatile in aerial parts and hexadecanoic acid (22.5%) in roots. MSD-SPME identified 3-ethyl-3,4-dihydro-2(1H)-quinoxalinone as the major shared volatile (9.8% in aerial parts; 14.5% in roots). Linoleic acid was the predominant fatty acid in both parts (26.4% in aerial parts; 22.3% in roots). LC-HRMS highlighted rutin (525.29 mg/100g $_{\text{dry plant}}$) in aerial parts and chlorogenic acid (24.78 mg/100g $_{\text{dry plant}}$) in roots as dominant phenolics. The 70% methanol aerial extract showed the strongest antioxidant activity (DPPH IC_{50}: 0.14 ± 0.02 mg/mL; CUPRAC: 375.1 ± 12.2 mM Trolox), while the highest TEAC value was recorded in the chloroform root extract (1.27 ± 0.02 mM). The same methanol extract also demonstrated the most potent α-amylase inhibition (IC_{50}: 0.537 ± 0.042 mg/mL). ICP-OES analysis revealed calcium as most abundant in aerial parts (24.9 mg/g) and potassium in roots and flowers (11.7 and 19.0 mg/g). These findings provide a detailed chemical and bioactivity profile of *C. kirikkaleensis*, supporting its potential for future pharmacological investigations.

Keywords: *Campanula kirikkaleensis*, phytochemical profile, antioxidant activity, LC-HRMS, GC-FID/MS

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1 Introduction

The utilization and recognition of medicinal plants vary significantly across regions, shaped by ecological diversity, cultural traditions, and local healthcare demands. Globally, phytotherapy remains an essential pillar of traditional medicine, particularly in regions such as Asia, Africa, and Latin America. Türkiye, owing to its remarkable biodiversity and strategic biogeographical position, hosts over 9,000 plant taxa, more than 30% of which are endemic—placing the country among the richest sources of medicinal flora and ethnobotanical knowledge in the world (Şenkul & Kaya, 2017; Pironon et al., 2024; Ambarlı et al., 2016).

The genus *Campanula* L., belonging to the Campanulaceae family, is well-represented in Türkiye with 136 recognized taxa, including recently described species. Notably, over half of these taxa are endemic (Liveri et al., 2019; Ozcan & Eminagaoglu, 2018). Ethnobotanical research suggests that members of this cosmopolitan genus—distinguished by their characteristic blue, bell-shaped flowers, have been traditionally employed in the treatment of various ailments (Erdoğan et al., 2025; Bussmann et al., 2016). Tsiftsoglou et al. (2023) reported that *C. pelviformis* is a rich source of dietary minerals, while Mok et al. (2023) demonstrated the therapeutic potential of *C. takesimana* in managing asthma, tonsillitis, and atopic dermatitis through modulation of cytokine-induced epidermal differentiation and lipid metabolism.

Several phytochemicals reported in *Campanula* species, including flavonoids (e.g., luteolin, apigenin), phenolic acids (e.g., caffeic acid, rosmarinic acid), and terpenoids, have

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demonstrated promising bioactivities in previous studies (Erdoğan et al., 2025). These compounds are well-known for their potent antioxidant and anti-inflammatory effects, which are highly relevant in preventing or mitigating oxidative stress-related disorders such as cardiovascular diseases, neurodegenerative conditions, diabetes mellitus, and certain cancers. Moreover, the inhibition of enzymes such as α-amylase is a well-recognized therapeutic target in the management of type 2 diabetes, suggesting that enzyme-inhibitory plant constituents may offer potential for antidiabetic drug development (Erdoğan et al., 2025). Therefore, identifying and characterizing the bioactive constituents of *C. kirikkaleensis* may provide novel leads for future biomedical applications.

Despite their extensive traditional use, comprehensive phytochemical and biological evaluations remain limited for many *Campanula* species, primarily due to their taxonomic complexity and wide geographic distribution. Furthermore, new species continue to be described. One such species is *Campanula kirikkaleensis*, first identified by Dönmez and Güner (1993) (World Flora Online (WFO), 2025). To date, scientific knowledge about *C. kirikkaleensis* remains scarce. Its selection was driven not only by its strict endemism to Kırıkkale Province but also by the documented ethnopharmacological relevance of closely related *Campanula* species, traditionally used for inflammatory, respiratory, and digestive ailments. Investigating this underexplored taxon may reveal novel bioactive compounds and support biodiversity conservation efforts. This species is narrowly endemic to Kırıkkale Province, Türkiye, typically found at elevations around 1,000 m above sea level (Urker, 2021).

Given the documented ethnopharmacological relevance and known bioactivities of related *Campanula* species, this study aims to comprehensively characterize the phytochemical profile of *C. kirikkaleensis* and evaluate its antioxidant and enzyme inhibitory properties through cell-free assays. This integrated approach is intended to provide scientific support for the pharmacognostic potential of this narrowly endemic species.

To date, no comprehensive phytochemical or bioactivity-oriented investigation has been reported for *C. kirikkaleensis*, and the literature lacks data on its essential oil profile, volatile composition (MSD-SPME), fatty acid composition, silylated metabolites, LC-HRMS phenolic profile, and cell-free antioxidant and α-amylase inhibitory activities. Unlike earlier studies focusing on other *Campanula* taxa, the present work provides the first multi-platform chemical characterization of this endemic species, integrating GC-FID/MS, MSD-SPME, LC-HRMS, fatty acid profiling, and mineral–vitamin analysis within a single framework. This holistic approach not only reveals several previously unreported metabolites, including the dominance of 3-ethyl-3,4-dihydro-2(1H)-quinoxalinone, but also demonstrates the species' distinct chemotaxonomic features and notable biological potential. These aspects clearly distinguish the current study from all formerly published data on the genus.

2 Materials and Methods

2.1 Plant Material Collection and Identification

Specimens of *C. kirikkaleensis* were collected in July 2022 from the vicinity of Sarmaşa Village Road in Çorum, Türkiye (elevation: 900 m; latitude: 40.3833° N; longitude: 34.5325° E), an area characterized by salt-rich (halophytic) and gypsum-bearing steppe habitats. Botanical identification was conducted by Dr. Ömer Koray YAYLACI (Department of Pharmaceutical Botany, Faculty of Pharmacy, Anadolu University, Eskişehir, Türkiye; https://avesis.anadolu.edu.tr/okyaylaci), based on the original taxonomic description provided by Dönmez and Güner (1993). A taxonomically verified specimen was deposited at the Herbarium of the Faculty of Pharmacy, Anadolu University (ESSE No: 15898) (Figures 1A–1C). The collected plant material was shade-dried under cool, well-ventilated conditions and subsequently separated into aerial parts and roots. Each part was then cut into appropriate sizes for phytochemical and cell-free activity evaluations.

2.2 Essential Oil Isolation by Hydrodistillation

The essential oils from *C. kirikkaleensis* were isolated by hydrodistillation of the herb and the roots (separately) using a Clevenger-type apparatus (IldamCam, Türkiye) for 3 hours. For each distillation, 50 g of dried plant material was placed in a 1000 mL round-bottom flask with 500 mL of distilled water. Hydrodistillation was chosen as a classical and widely accepted method for isolating volatile essential oils, allowing comparison with other *Campanula* species in the literature (European Directorate for the Quality of Medicines & HealthCare, 2023). The collected oils were stored in amber glass vials at 4°C to prevent photodegradation. The essential oil yield was calculated as % w/w based on the dry weight of the plant.

2.3 Extraction of Volatile Compounds

Since the plant is very poor in essential oil, the volatile compounds were investigated by Micro-Steam Distillation–Solid Phase Microextraction (MSD-SPME) technique in order to directly capture the volatile compounds. MSD-SPME was chosen as a highly sensitive, solvent-free technique that enables efficient extraction and direct analysis of low-abundance volatile compounds, providing complementary information to conventional hydrodistillation and overcoming its limitations in plant species with very low essential oil content (Çetin, 2025). For each extraction, 0.5 g of the aerial parts and roots separately were placed in 25 mL glass flasks containing 3 mL of distilled water. The mixture was gently heated, and distillation-extraction was carried out simultaneously for 3 minutes using a polydimethylsiloxane/divinylbenzene (PDMS-DVB) fiber (Supelco, Merck KGaA, Darmstadt, Germany). After completion of the extraction process, the fiber was directly subjected to analysis by Gas Chromatography–Mass Spectrometry GC-FID/MS (Agilent 6890N; SEM Ltd, Istanbul, Türkiye) (Çetin, 2025).

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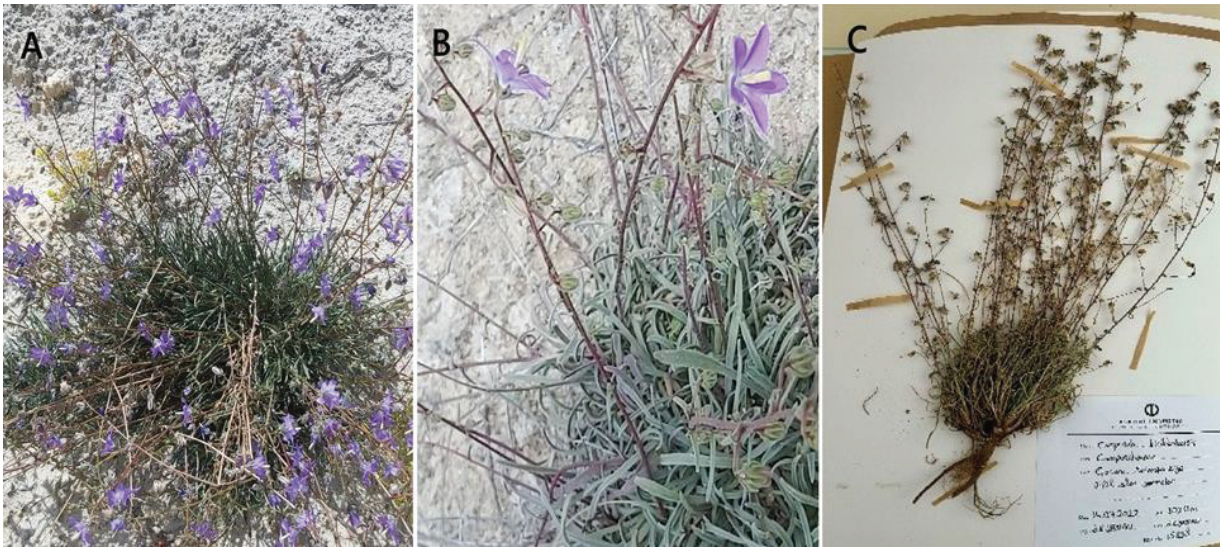

Figure 1. *Campanula kirikkaleensis*. (A) Natural habitat and overall appearance of *Campanula kirikkaleensis* in the wild; (B) Close-up view of its characteristic blue, bell-shaped flowers; (C) Herbarium specimen of *C. kirikkaleensis* (ESSE No: 15898)

2.4 Solvent Extraction

The aerial parts and roots of *C. kirikkaleensis* were separately extracted at room temperature by maceration using three different solvents: *n*-hexane (C_6H_{14}; Merck, Germany), chloroform ($CHCl_3$; Sigma-Aldrich, St. Louis, MO, USA), and 70% methanol (CH_3OH; Darmstadt, Germany). For each extraction, 10 g of plant material was mixed with 200 mL of solvent. Extractions were performed on a shaker at 110 rpm for 48 hours. After extraction, the mixtures were filtered using pleated filter paper and the solvents were evaporated under reduced pressure at 40°C using a rotary evaporator (Heidolph Instruments, Germany). The extracts obtained were stored under appropriate conditions to be used in further studies. In addition to these independent extractions, sequential solvent extraction was also carried out for both aerial and root materials. After maceration with n-hexane and filtration, the residual plant material was dried under a gentle stream of nitrogen to remove remaining traces of the solvent. The same plant material was then successively macerated with chloroform and finally with 70% methanol, following the same procedure described above. Thus, sequential extraction was performed using solvents of increasing polarity.

2.5 Silylation of Extracts

To determine the chemical composition of the extracts, silylation was carried out prior to GC-FID/MS analysis to derivatize polar, thermolabile, and non-volatile compounds, rendering them volatile and thermally stable for accurate GC analysis. Specifically, 2.0 mg of each extract was mixed with 70 µL of pyridine and 70 µL of N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% trimethylchlorosilane (TMCS). The mixtures were heated in a sand bath at 100°C for 1 hour to complete derivatization.

2.6 Microextraction of Fixed Fatty Acids

Fixed fatty acids were extracted from 0.2 g of the root and aerial parts using a commercial Fatty Acid Extraction Kit. (Sigma-Aldrich, St. Louis, MO, USA). The plant materials were homogenized in 3.0 mL of extraction solution with a tissue homogenizer (15,000 rpm, 5 min). Transesterification was subsequently performed under a nitrogen atmosphere. For derivatization, 100 µL of boron trifluoride (BF_3) reagent and 300 µL of *n*-hexane were added to the homogenate, followed by heating at 95°C for 1 hour in a sand bath. After cooling, 1 mL of *n*-hexane and 1 mL of distilled water were added, and the mixture was vortexed and then allowed to stand for phase separation. Then, the hexane phase was separated by using pasteur pipette. Prior to gas chromatographic analysis, the samples were concentrated under a gentle stream of nitrogen gas (Möller et al., 2019). This protocol was applied to extract and derivatize fixed fatty acids from the aerial parts and roots, enabling accurate GC-FID/MS analysis for comprehensive identification and quantification of the plant's fatty acid composition.

2.7 Mineral and Vitamin Analysis

In addition to the aerial and root parts, flowers were also collected, dried, and analyzed for mineral content using the same ICP–OES procedure described above. Plant samples were digested with concentrated nitric acid using a microwave-assisted digestion system and subsequently diluted with distilled water. Mineral content was quantified using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) for simultaneous multi-element detection, including Zn, Cd, Co, Ni, Fe, Mg, Ca, Cu, Na, and K. Results were expressed as milligrams of element per gram of dry plant material (mg/g) (Çetin, 2025).

In addition, the lipophilic vitamin profile—including vitamin K_2, vitamin E, vitamin E acetate, vitamin A palmitate, and β-carotene—was determined using Ultra Performance

Convergence Chromatography (UPC^2; Waters Acquity, Milford, MA, USA) coupled with a Photodiode Array (PDA) detector (European Directorate for the Quality of Medicines & HealthCare, 2023). Prior to analysis, the samples were dissolved in methyl tert-butyl ether (MTBE). This approach was employed to accurately quantify essential mineral elements and lipophilic vitamins in the plant, providing a comprehensive nutritional and bioactive profile while ensuring high sensitivity and reproducibility of the measurements.

2.8 Cell-Free Antioxidant and Enzyme Inhibition Assays

The antidiabetic and antioxidant potentials of *C. kirikkaleensis* were evaluated through a series of cell-free bioassays. Antidiabetic activity was assessed via inhibition of pancreatic α-amylase, with absorbance measured at 630 nM using a microplate reader (Zengin et al., 2014). Antioxidant activity was determined using three spectrophotometric methods:

(i) The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay (Sigma-Aldrich, St. Louis, MO, USA) (Krishnaiah et al., 2011);
(ii) The cupric ion reducing antioxidant capacity (CUPRAC) assay, which quantifies the reduction of Cu^{2+} to Cu^{+} ions (Özyürek et al., 2011); and,
(iii) The Trolox Equivalent Antioxidant Capacity (TEAC) assay based on the scavenging of the $ABTS^{+\bullet}$ radical cation (2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid)) (Re et al., 1999).

These assays were selected to provide a comprehensive, cell-free evaluation of the antioxidant and enzyme inhibitory properties of the extracts, enabling an accurate and controlled assessment of their radical-scavenging and α-amylase inhibitory activities without the confounding effects of cellular metabolism.

2.9 Gas Chromatography–Mass Spectrometry (GC–MS) Analysis

GC–MS analysis was performed according to previously described conditions (Özek et al., 2006). An Agilent HP-Innowax FSC capillary column (60 m × 0.25 mM, 0.25 µM film thickness) was used with helium as the carrier gas at a constant flow rate of 0.8 mL/min. The oven temperature was initially held at 60°C for 10 minutes, then raised to 220°C at a rate of 4°C/min, held for 10 minutes, and finally increased to 240°C at 1°C/min. The injection was performed in split mode with a split ratio of 40:1, and the injector temperature was set at 250°C. Mass spectra were recorded in electron impact (EI) mode at 70 eV over a mass range of 35–450 *m/z*. Compound identification was performed by comparing the obtained mass spectra with reference data from the Wiley GC/MS Library (Wiley, New York, NY, USA) (Wiley Science Solutions, 2024), Joulain et al. (2001), the Adams Library (Adams, 2005), the Başer Library (Başer & Demirci, 2007), and Ozek et al. (2007).

2.10 Gas Chromatography–Flame Ionization Detection (GC–FID) Analysis

GC–FID analysis was conducted using an Agilent 6890N GC system. To ensure the same elution order as in the GC–MS analysis, the effluent was split between the FID and MS detectors, and a single injection was performed using the same capillary column and identical analytical conditions. The FID temperature was set at 300°C.

2.11 Analysis of Silylated Compounds

For the analysis of silylated derivatives prepared from *n*-hexane, 70% methanol, and chloroform extracts, an Agilent 7890A GC system coupled with a 5975C Inert Mass Selective Detector (MSD) equipped with a Triple-Axis detector was employed. Chromatographic separation was achieved using an HP-5 FSC capillary column (30 m × 0.25 mM, 0.25 µM film thickness; Agilent, Walt & Jennings Scientific, Wilmington, DE, USA). Helium was used as the carrier gas at a constant flow rate of 0.8 mL/min. The oven temperature was programmed to increase from 100°C to 320°C at a rate of 3°C/min and was held at 320°C for 16.67 minutes. The injector temperature was maintained at 250°C, while the flame ionization detector (FID) temperature was set at 300°C. The injection was performed in splitless mode. 1.0 µL of each derivatized sample was directly injected into GC-FID/MS systems. Mass spectra of the separated compounds were recorded in electron impact (EI) mode at 70 eV, within the mass range of 35–1050 *m/z*. Compound identification was based on comparison of retention times and mass spectral data with the Wiley GC/MS Library (Wiley, New York, NY, USA). Additionally, the "Special Özek Silyl Derivative Library," developed from standard compound derivatives, was used as a reference database. Quantitative analysis was performed by calculating the relative percentage areas of the peaks detected by the FID (Çetin, 2025; Razboršek et al., 2008).

2.12 Analysis of Phenolic Compounds

Phenolic constituents in the extracts were analyzed using a liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS) system (Thermo ORBITRAP Q-Exactive) equipped with an electrospray ionization (ESI) source. Chromatographic separation was achieved on a Troyasil C_{18} HS column (150 × 3 mM, 3.5 µM) at a flow rate of 0.35 mL/min. The mobile phases consisted of water with 1% formic acid (A) and methanol with 1% formic acid (B). The gradient program initiated at 50% B, increased to 100% at 3 min, was held constant until 6 min, returned to 50% at 7 min, and maintained this level until 15 min.

Mass spectra were acquired over an *m/z* range of 100–900 under optimized ionization conditions. The ion source parameters were as follows: sheath gas flow rate, 45; auxiliary gas flow rate, 10; spray voltage, 3.80 kV; capillary temperature, 320°C; auxiliary gas heater temperature, 320°C; and S-lens RF level, 50. The mass resolution was set to 70,000 at m/z 200. Compound identification was conducted by comparison with entries in the ILMER spectral library (Bezmialem Vakıf University). Environmental conditions

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Table 1. Solvent-specific extraction efficiencies of *C. kirikkaleensis* aerial and root parts

Extract type	Extract code	Yield*, %
Aerial part essential oil	Ap-EO	0.096
Root essential oil	R-EO	0.031
n-Hexane extract of the aerial part	Ap-HE	1.3
Chloroform extract of the aerial part	Ap-CE	2.0
Methanol extract of the aerial	Ap-ME	13.6
n-Hexane extract of the root	R-HE	1.3
Chloroform extract of the root	R-CE	3.3
Methanol extract of the root	R-ME	17.5
Chloroform extract of the aerial part following *n*-hexane extraction	Ap-HCE	0.7
Chloroform extract of the root following *n*-hexane extraction	R-HCE	0.8
70% methanol extract of the aerial following sequential *n*-hexane and chloroform extraction	Ap-HCME	11.0
70% methanol extract of the root following sequential *n*-hexane and chloroform extraction	R-HCME	14.5

Note: *Yields were calculated based on the air-dried weight of the plant material and expressed as % w/w.

during analysis were maintained at 22.0 ± 5.0°C and 50 ± 15% relative humidity.

3 Results

Within the scope of this study, the phytochemical composition and cell-free antioxidant and enzyme inhibition potential of *C. kirikkaleensis* were comprehensively evaluated. Maceration with n-hexane, chloroform, and 70% methanol under continuous shaking (110 rpm) at room temperature yielded extracts that were subsequently filtered through pleated filter paper. Extraction efficiency was expressed as the percentage of extract mass relative to the dry weight of the plant material. The highest yields were determined in the root methanol extract (R-ME; 17.5% w/w), followed by the sequential root extract (R-HCME; 14.5% w/w), the aerial part methanol extract (Ap-ME; 13.6% w/w), and the sequential aerial part hexane, chloroform and methanol extract (Ap-HCME; 11.0% w/w).

The relatively high extraction efficiencies obtained from the methanol extracts—particularly from the root—suggest a higher concentration of polar phytoconstituents in this plant part. A detailed summary of extraction yields is provided in Table 1. A comprehensive set of supplementary tables (S1–S4) provides detailed phytochemical compositions, chromatographic results, and compound-specific data supporting the findings presented in this study.

3.1 Essential Oil Chemical Composition

The essential oil obtained from the aerial part of *C. kirikkaleensis* contained 63 compounds, representing 96.5% of the total oil content. The major constituents were phytol (14.2%), pentacosane (12.8%), and heptacosane (9.2%). In terms of chemical classification, the essential oil from the aerial parts was predominantly composed of long-chain hydrocarbons, along with notable contributions from sesquiterpenes, aldehydes, fatty alcohols, norisoprenoids (e.g., β-ionone derivatives), and oxygenated compounds such as phytol, indicating a chemically rich and structurally varied profile. In the root oil, 52 compounds were identified, accounting for 97.5% of the total composition. The primary constituents were hexadecanoic acid (22.5%), 3-ethyl-3,4-dihydro-2(1H)-quinoxalinone (20.6%), and triacontane (8.0%). These results indicate that the root oil of *C. kirikkaleensis* is rich in long-chain fatty acids, hydrocarbons, and heterocyclic structures, reflecting its complex chemical makeup. A complete list of all identified volatile constituents, along with their relative abundances and retention indices, is provided in Supplementary Table S1.

3.2 Volatile Compound Profiles Obtained by MSD-SPME Analysis

Based on the results of MSD-SPME analysis, a total of 59 volatile compounds were identified in the aerial parts of *C. kirikkaleensis*. The major constituents were 3-ethyl-3,4-dihydro-2(1H)-quinoxalinone (9.8%), hexadecanoic acid (7.8%), and (*E*)-β-ionone (5.5%). In the root extracts, 57 compounds were detected, with the principal constituents being 3-ethyl-3,4-dihydro-2(1H)-quinoxalinone (14.5%), hexanol (10.7%), (*E*,*E*)-2,4-decadienal (10.1%), and (*E*,*Z*)-2,4-decadienal (7.1%). Notably, 3-ethyl-3,4-dihydro-2(1H)-quinoxalinone was the most abundant volatile compound in both plant parts.

All volatile constituents occurring at ≥3% are summarized in Table 2. The complete list of all identified volatile compounds—including both major and minor constituents—along with their retention indices and relative abundances, is provided in Supplementary Table S2.

3.3 Phytochemical Profile of Silylated Derivatives

The compounds identified in the silylated (TMS) derivatives of *C. kirikkaleensis* aerial part and root extracts, as determined by GC-FID/MS analyses, are summarized in Supplementary Tables S3–S4. The analyses revealed distinct phytochemical profiles between the two plant parts and among different solvent fractions. In the aerial part extracts, octacosanoic acid and β-sitosterol were predominant in both the hexane (16.8% and 12.0%, respectively) and chloroform (20.0% and 11.3%, respectively) fractions. The

Table 2. Major volatile constituents of *C. kirikkaleensis* identified by MSD-SPME analysis

RRI	Compound	Aerial part %	Root %
1093	Hexanal	–	4.3
1360	Hexanol	0.2	10.7
1399	Nonanal	0.9	3.5
1523	(*E,Z*)-3,5-Octadien-2-one	–	4.7
1779	(*E,Z*)-2,4-Decadienal	–	7.1
1827	(*E,E*)-2,4-Decadienal	1.5	10.1
1868	(*E*)-Geranyl acetone	4.8	1.6
1958	(*E*)-β-Ionone	5.5	–
2037	3-Ethyl-3,4-dihydro-2(1H)-quinoxalinone #	9.8	14.5
2131	Hexahydrofarnesyl acetone	4.7	0.3
2178	3,4-Dimethyl-5-pentylidene-2(5*H*)-furanone	5.2	–
2239	Carvacrol	4.6	0.6
2343	2-Ethylhexyl salicylate	–	7.0
2629	*MW:276, BP:121*	5.5	–
2670	Tetradecanoic acid	3.8	–
2935	Hexadecanoic acid (=*Palmitic acid*)	7.8	1.1

Note: RRI: Relative Retention Index was calculated for the polar column with n-alkanes (C7–C40); %: Calculated from the FID chromatogram; #: Identified based on spectral similarity with the Wiley-NIST library; MW: Molecular weight; BP: Base peak.

70% methanol extract of the aerial part was characterized by high levels of sucrose (20.8%) and quinic acid (4.9%). In the combined hexane and chloroform extracts, methyl-butyl-α-D-mannopyranoside (26.3%) and octacosanoic acid (18.0%) were the major constituents, while the sequential hexane, chloroform, and methanol extract was dominated by sucrose (46.3%) and *myo*-inositol (11.7%).

Similarly, the root extracts displayed distinct profiles. In the hexane extract, 1-octacosanol (12.6%) and β-sitosterol (10.5%) were predominant, whereas the chloroform extract contained octacosanoic acid (18.9%) and 1-octacosanol (15.6%) as major constituents. The 70% methanol extract of the root was notably rich in sucrose (52.9%). In the combined hexane and chloroform root extract, octacosanoic acid (16.6%) and hexacosanoic acid (14.0%) were most abundant, while the sequential hexane–chloroform–methanol extract contained high levels of 5-hydroxymethylfurfural (9.7%) and trimethyl((4-methylcyclohexyl)methoxy)silane (7.9%).

3.4 Fatty Acid Composition and Omega-Series Distribution in C. kirikkaleensis

The fixed fatty acid content of *C. kirikkaleensis* was analyzed through fatty acid methyl esters (FAMEs), using gas chromatography–mass spectrometry (GC-MS) for qualitative identification and gas chromatography with flame ionization detection (GC-FID) for quantification. In the aerial part extract, 11 fatty acids were identified, with linoleic acid (26.4%), palmitic acid (17.4%), and α-linolenic acid (13.0%) as the predominant constituents. Similarly, the root extract contained 11 fatty acids, among which linoleic acid (22.3%), hexacosanoic acid (21.6%), and nonadecanoic acid (14.8%) were the most abundant. The FAME profiles of both plant parts are detailed in Table 3. Additionally, the unsaturated fatty acids were classified by their omega (ω) series, revealing a predominance of ω-6 and ω-3 fatty acids, both of which are essential for maintaining cellular function, membrane fluidity, and overall metabolic health.

3.5 Mineral Profile and Vitamin Screening of C. kirikkaleensis

Results of the mineral and vitamin analyses of *C. kirikkaleensis* aerial and root parts revealed distinct outcomes. The fat-soluble vitamin profile was assessed using Ultra Performance Convergence Chromatography (UPC^2), targeting vitamins K_2, E, E acetate, A palmitate, and β-carotene. However, none of these vitamins were detected in the aerial part or root samples under the applied analytical conditions. Mineral composition was determined using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). The most abundant elements were calcium (24.9 mg/g) in the aerial part, potassium (11.7 mg/g) in the roots, and potassium (19.0 mg/g) in the flowers.

3.6 Evaluation of Antioxidant and Enzyme Inhibitory Activities

The cell-free antioxidant and enzyme inhibitory activities of *C. kirikkaleensis* extracts were evaluated using assays, including free radical scavenging (DPPH, TEAC/$ABTS^{\bullet+}$, and CUPRAC) and pancreatic α-amylase inhibition tests, to assess their antioxidant and antidiabetic potential. A comparative summary of the antioxidant and α-amylase inhibitory effects of the extracts is provided in Figure 2.

3.6.1 Results of Antioxidant Activity Assays

The antioxidant potential of *C. kirikkaleensis* extracts was assessed using three cell-free in vitro methods. DPPH, TEAC ($ABTS^{\bullet+}$), and CUPRAC. Each method reflects a different

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Table 3. Fatty Acid Methyl Ester (FAME) composition of *C. kirikkaleensis* analyzed by GC-FID/MS

No	RRI	Compound	Aerial part %	Root %
1	2016	Methyl myristate (14:0)	0.7	11.3
2	2223	Methyl palmitate (16:0)	17.4	–
3	2330	Methyl heptadecanoate (17:0)	–	1.0
4	2436	Methyl stearate (18:0)	2.1	1.9
5	2468	Methyl oleate (18:1) **ω-9**	7.5	4.1
6	2509	Methyl linoleate (18:2) **ω-6**	26.4	22.3
7	2542	Methyl nonadecanoate (19:0)	11.8	14.8
8	2572	Methyl linolenate (19:3) **ω-3**	13.0	6.0
9	2642	Methyl arachidate (20:0)	0.8	2.0
10	2843	Methyl behenate (22:0)	1.8	7.3
11	3050	Methyl lignocerate (24:0)	7.3	7.6
12	3280	Methyl hexacosanoate (26:0)	11.1	21.6
		Total	**99.9**	**99.9**
		Total ω-3	13.0	6.0
		Total ω-6	26.4	22.3
		Total ω-9	7.5	4.1
		Total unsaturated fatty acids	**46.9**	**32.4**

Note: RRI: Relative Retention Index was calculated for the polar column with n-alkanes (C_7–C_{40}).

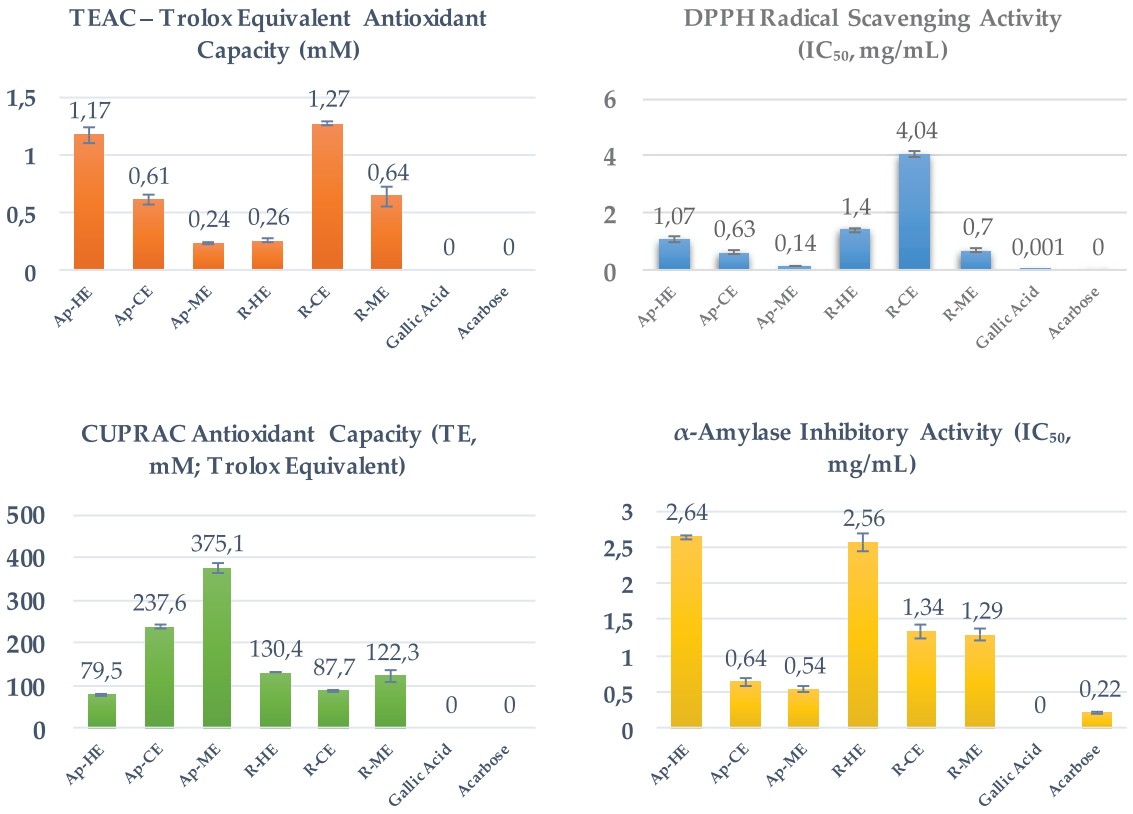

Figure 2. Antioxidant and α-Amylase inhibitory activities of *C. kirikkaleensis* extracts

mechanism of antioxidant action, such as radical scavenging or electron transfer capacity. In the DPPH assay, which measures the ability to neutralize free radicals via hydrogen atom donation, the 70% methanol extract of the aerial part exhibited the highest activity with an IC_{50} value of 0.14 ± 0.02 mg/mL, indicating strong radical scavenging capacity. Gallic acid was used as the reference antioxidant. The TEAC assay, based on the scavenging of the $ABTS^{\bullet+}$

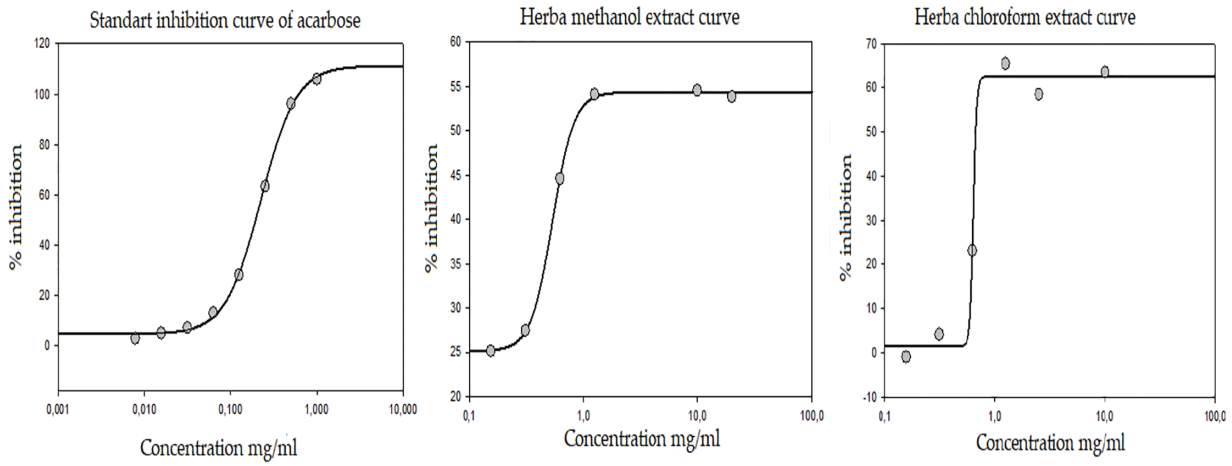

Figure 3. Dose–response inhibition curves of pancreatic α-amylase by (A) acarbose (standard), (B) 70% methanol extract of C. herba, and (C) chloroform extract of herba

radical cation, revealed the chloroform extract of the root to be the most effective, showing a Trolox equivalent antioxidant capacity of 1.27 ± 0.02 mM. This value was derived from a Trolox calibration curve with excellent linearity (r^2 = 0.9951), confirming the reliability of the measurements. In the CUPRAC assay, which evaluates the ability of antioxidants to reduce Cu^{2+} to Cu^{+} in the presence of neocuproine, the strongest response was again observed in the 70% methanol extract of the aerial part. This sample exhibited a copper-reducing capacity of 375.1 ± 12.2 mM TE, as determined from a standard curve with an excellent correlation (r^2 = 0.9999). All measurements were performed in triplicate and expressed as means ± SD. A summary of the antioxidant activity results is provided in Figure 2.

3.6.2 α-Amylase Inhibitory Activity for Antidiabetic Evaluation

Based on the IC_{50} value of the standard inhibitor acarbose (0.22 ± 0.01 mg/mL), the strongest α-amylase inhibitory activity among the *C. kirikkaleensis* extracts was observed in the 70% methanol extract of the aerial part (0.54 ± 0.04 mg/mL), followed by the aerial part chloroform extract (0.64 ± 0.05 mg/mL) (Figures 3A–3C). In contrast, the root extracts exhibited weaker inhibition, with IC_{50} values averaging around 1.2 mg/mL (Figure 3). The dose–response inhibition curves of the remaining extracts are provided in the Supplementary Materials (Figures S1–S4) for reference.

3.7 Phenolic Compounds

In the 70% aqueous methanol extracts of *C. kirikkaleensis*, rutin (525.29 mg/100 g $_{\text{dry weight}}$) was identified as the major phenolic compound in the aerial part, while chlorogenic acid (24.78 mg/100 g $_{\text{dry weight}}$) predominated in the root. Detailed results are presented in Table 4. These findings reveal distinct phenolic distributions between plant parts and suggest that the rutin- and chlorogenic acid-rich fractions may contribute to the antioxidant properties observed in the cell-free assays.

4 Discussion

To the best of our knowledge, this is the first study to investigate the phytochemical composition and cell-free antioxidant and enzyme inhibitory properties of *C. kirikkaleensis* Dönmez & Güner, an endemic species for which no prior data on chemical constituents or bioactivity have been reported in the literature. The present study comprehensively profiled the phytochemical constituents and cell-free antioxidant and enzyme inhibitory properties of *C. kirikkaleensis*. Based on the hypothesis that this endemic species harbors biologically active compounds similar to related *Campanula* taxa, our findings provide clear evidence of its pharmacognostic potential, highlighting the relevance of its unique phytochemical composition and bioactivity for further therapeutic exploration.

In the essential oil composition, phytol (14.2%), pentacosane (12.8%), and heptacosane (9.2%) were identified as the major components of the aerial part, while the root oil was rich in hexadecanoic acid (22.5%), quinoxalinone (20.6%), and triacontane (8.0%). MSD-SPME analysis revealed 3-ethyl-3,4-dihydro-2(1H)-quinoxalinone to be the most abundant volatile in both aerial and root parts, reaching 14.5% in the root. The silylated extracts of the aerial part predominantly contained octacosanoic acid, β-sitosterol, sucrose, and quinic acid, while the root fractions featured 1-octacosanol, octacosanoic acid, hexacosanoic acid, and high levels of sucrose (up to 52.9%). In terms of fatty acid content, linoleic acid was the most abundant in both aerial part (26.4%) and root (22.3%), followed by palmitic acid, α-linolenic acid, and nonadecanoic acid. No fat-soluble vitamins were detected, whereas calcium (24.9 mg/g) was the most concentrated mineral in the aerial part and potassium in both root (11.7 mg/g) and flower (19.0 mg/g) parts. Among the phenolic compounds, rutin (525.29 mg/100 g $_{\text{dry weight}}$) was dominant in the aerial part and chlorogenic acid (24.78 mg/100 g $_{\text{dry weight}}$) in the root. Regarding cell-free antioxidant activity, the 70% methanol extract of the aerial part exhibited the highest

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Table 4. Phenolic profiles of *Campanula kirikkaleensis* extracts determined by LC-HRMS

No	Analytes	RT[a]	m/z[b]	RU (%)	Quantitative results (mg analyte/100g $_{\text{dry plant}}$)	
					Ap-ME	R-ME
1	Chlorogenic acid	8.35	353.0860	3.58	104.10	24.78
2	Fumaric acid	7.72	115.0031	2.88	12.08	4.37
3	Caffeic acid	8.69	179.0341	3.74	7.93	3.19
4	Luteolin-7-rutinoside	9.19	593.1482	3.06	78.81	0.15
5	Vanilic acid	9.09	167.0341	3.49	40.43	9.98
6	p-Coumaric acid	8.42	163.0392	3.31	26.69	9.44
7	Rutin	8.94	609.1430	3.07	525.29	4.08
8	Syringic acid	9.64	197.0446	3.71	21.31	11.42
9	Hyperoside	8.69	179.0341	3.46	69.92	N.D.
10	Quercitrin	9.02	463.0858	3.78	19.11	N.D.
11	Scutellarein	10.37	285.0390	2.84	2.47	N.D.
12	Quercetin	9.83	301.0338	2.95	2.97	N.D.
13	Salicylic acid	10.22	137.0237	1.89	1.20	0.71

Note: RT[a]: Retention time, *m/z*: mass-to-charge ratio of the detected ion., Ap-ME: Aerial part %70 methanol extract, R-ME: Root %70 methanol extract, RU: Relative Uncertainty, N.D.: Not detected.

activity in DPPH (IC_{50}: 0.14 ± 0.02 mg/mL) and CUPRAC (375.1 ± 12.2 mM TE) assays, while the chloroform extract of the root showed the highest TEAC activity (1.27 ± 0.02 mM TE). The same methanol extract of the aerial part also demonstrated the strongest α-amylase inhibitory effect (IC_{50}: 0.537 ± 0.042 mg/mL).

Campanula species are known to produce extracts in varying yields depending on plant part and solvent polarity (Marah et al., 2024). In the present study, extract yields exceeding 10% were obtained from both the 70% methanol and the sequential *n*-hexane–chloroform–methanol extracts of the aerial part and root of *C. kirikkaleensis*. These yields were sufficient to enable detailed phytochemical profiling and cell-free biological activity assessments. The observed differences in extract yields among *Campanula* species, including the relatively higher yield of *C. kirikkaleensis* compared to *C. baskilensis* and *C. glomerata*, and its lower yield than *C. takesimana* (Marah et al., 2024; Sarikurkcu et al., 2017; Kim et al., 2012), may be influenced by several factors. These include intrinsic variations in phytochemical density, differences in plant tissue architecture, and the phenological stage at the time of harvest. Additionally, solvent polarity, extraction duration, and methodology—such as maceration versus Soxhlet or ultrasonic extraction—can significantly impact extract recovery. The specific plant part used (aerial part vs. root), environmental conditions, and genetic variability among species are also known to play crucial roles in determining overall extractive efficiency.

Essential oils (EO), valued for their pleasant aroma and high content of bioactive constituents, have gained growing interest not only in traditional applications such as perfumery and flavoring, but also in modern medical and therapeutic contexts. Their demonstrated antimicrobial, antioxidant, anti-inflammatory, and analgesic properties have supported their incorporation into a wide range of pharmaceutical, cosmetic, and food preservation products (Ramsey et al., 2020; Pezantes-Orellana et al., 2024). The chemical complexity of essential oils derived via hydrodistillation is strongly influenced by the distillation parameters, plant matrix characteristics, and the sensitivity of the analytical techniques employed—particularly gas chromatography coupled with mass spectrometry (GC-MS) or flame ionization detection (GC-FID) (De Groot & Schmidt, 2016a; Gibbs, 2019). In the present study, although the EO yields of both the aerial part (0.096%) and root (0.031%) of *C. kirikkaleensis* remained below 1%, as is typical for many *Campanula* species (Tosun et al., 2011; Salajeghe et al., 2015), a total of 85 EO compounds were identified across both parts (Supplementary Table S1), reflecting a remarkable degree of chemical richness despite the relatively low yield. Phytol, which was identified as the major constituent in the aerial part extract, and hexadecanoic acid, the most abundant compound in the root oil, are of particular interest due to their strong anti-inflammatory activity, as well as their roles in vitamin synthesis, anti-aging applications, and skin-protective formulations (Islam et al., 2018; Aparna et al., 2012). Future studies involving a greater number of samples and alternative extraction techniques may lead to the identification of additional essential oil components and a more comprehensive chemical profile (De Groot & Schmidt, 2016b).

The analysis of volatile constituents, similar to essential oils, represents a key approach for elucidating the therapeutic relevance and biological functions of medicinal plants (Mathe et al., 2024; Aziz et al., 2022). Unlike previous studies on other *Campanula* species, the volatile constituent profile of *C. kirikkaleensis* was investigated using the MSD-SPME technique, which allows for solvent-free extraction and enhanced sensitivity to low-abundance volatiles directly from plant matrices (Korkmaz et al., 2020; Özek et al., 2025). A comprehensive volatile profiling of *C. kirikkaleensis* yielded a total of 94 compounds, including 22 shared between the aerial and root parts, each exhibiting distinct abundance

profiles, along with one unidentified constituent (Table S2). Among these, 3-ethyl-3,4-dihydro-2(1H)-quinoxalinone, a quinoxaline derivative, emerged as the major volatile compound and is recognized for its antimicrobial, anti-inflammatory, and anticancer properties (Ramli et al., 2014; Ahmed et al., 2022). 2-Ethylhexyl salicylate, present at 7% in the root extract, is commonly utilized as a UVB filter in commercial sunscreen formulations (Pniewska & Kalinowska-Lis, 2024). Furthermore, hexanol, a volatile alcohol frequently found in essential oils, has demonstrated antimicrobial activity in food systems (Kyoui et al., 2023). Notably, 2,4-decadienal, identified at 10.1% in the root extract, has been shown to suppress gastric emptying rate and reduce energy intake (Kashima et al., 2021), suggesting that it may have potential relevance in the context of obesity-related research. In previous studies, the volatile constituents of *C. glomerata* subsp. *hispida* essential oil were analyzed using GC-FID/MS, and the major components were identified as hexadecanoic acid (24.51%), docosane (15.9%), isocitronellene (12.6%), heneicosane (4.6%), hexahydrofarnesyl acetone (3.2%), 9-tricosene (1.6%), octadecanol (1.4%), caryophyllene oxide (1.3%), α-funebrene (1.2%), and β-thujaplicinol (1.1%) (Sinek et al., 2012). Similarly, in *C. olympica*, the main volatile compounds were reported as 2E,6Z-farnesol (14.8%), 3,3-dimethyl-2-[5-methoxy-3-methyl-2-pentylidene]-1-cyclohexanone (12.1%), dehydroaromadendrane (11.6%), tetracosane (9.0%), pentacosane (7.9%), epoxy alloaromadendrene (5.9%), and cyclohexadecanolide (5.8%) (Tosun et al., 2011). For *C. kermanica*, the major volatile constituents were reported as caryophyllene oxide (23.3%), bornyl acetate (17.5%), o-menth-8-ene (11.6%), borneol (10.2%), and 1,8-cineole (10.0%) (Salajeghe et al., 2015). Compared to *C. glomerata subsp. hispida*, where hexadecanoic acid (24.51%) and long-chain alkanes such as docosane and heneicosane dominate the essential oil composition (Sinek et al., 2012), *C. kirikkaleensis* exhibited a partially overlapping fatty acid profile in its root oil (hexadecanoic acid as the major compound) but a distinctly different aerial part profile dominated by phytol, a diterpene alcohol with diverse bioactivities. Unlike *C. olympica*, which is characterized by sesquiterpenoid alcohols such as 2E,6Z-farnesol and macrocyclic lactones (Tosun et al., 2011), *C. kirikkaleensis* contained a quinoxaline derivative—3-ethyl-3,4-dihydro-2(1H)-quinoxalinone—as the major volatile, a compound not previously reported within the genus, suggesting that it may represent a potential chemotaxonomic marker. Similarly, *C. kermanica* showed monoterpene-rich profiles (borneol, bornyl acetate, and 1,8-cineole) (Salajeghe et al., 2015), which differ clearly from *C. kirikkaleensis*. Collectively, these interspecific differences likely reflect a combination of species-specific biosynthetic capacities, microhabitat-driven metabolic adaptation, and methodological disparities in extraction and analysis.

The literature on silylated compounds in extracts of the genus *Campanula* remains limited. Among sample preparation techniques for GC- FID/MS, silylation remains a key step in enabling the analysis of non-volatile, thermolabile, and polar compounds, thus providing deeper insight into the plant's metabolic composition and potential biological functions (Proestos & Komaitis, 2013). In our analysis, various silylated derivatives were successfully identified from the extracts of *C. kirikkaleensis*, reflecting the chemical diversity of its polar and semi-polar constituents. Sequential extraction using n-hexane, chloroform, and 70% methanol was employed prior to silylation to fractionate metabolites according to their polarity. This approach allowed the systematic separation of non-polar, moderately polar, and polar compounds, thereby minimizing matrix complexity and facilitating the comprehensive GC-FID/MS profiling of sugars, sugar alcohols, organic acids, and sterols that might otherwise remain undetected in single-step extractions. Octacosanoic acid and β-sitosterol, the predominant constituents obtained from the hexane and chloroform extracts of *C. kirikkaleensis*, have been reported to possess not only antitumor and antimicrobial properties but also anti-inflammatory, hypocholesterolemic, and hepatoprotective activities (Benramdane et al., 2022; Liu et al., 2023; Wang et al., 2023; Zhang et al., 2024). Other key constituents identified in *C. kirikkaleensis* extracts included 1-octacosanol, which has been reported to exhibit anti-fatigue, anti-hypoxic, antioxidant, anti-inflammatory, antitumor, cholesterol-lowering, and insecticidal activities (Marrero Delange & González Bravo, 2001; Zavala et al., 2020; Zhou et al., 2022). Quinic acid was also identified and is associated with antioxidant, antidiabetic, anticancer, antimicrobial, antiviral, anti-aging, cytoprotective, antinociceptive, and analgesic effects (Benali et al., 2022). Sucrose has been reported to inhibit bacterial growth (Nithish et al., 2021). Additionally, *myo*-inositol is recognized for its role in mitigating insulin resistance, depression, anxiety, polycystic ovary syndrome, and various metabolic and reproductive disorders (Dinicola et al., 2021). Additionally, the unidentified compound detected in our volatile profiling suggests that unexplored phytoconstituents may still be present in *C. kirikkaleensis*, indicating potential avenues for future studies employing complementary analytical techniques.

The fatty acid composition of *C. kirikkaleensis* revealed a diverse and abundant profile, particularly rich in essential polyunsaturated fatty acids such as linoleic (ω-6) and α-linolenic acid (ω-3), along with considerable levels of saturated and long-chain fatty acids, indicating its nutritional and pharmacological value (Table 3). Considering the therapeutic relevance of certain fatty acids—such as their anti-inflammatory, cardioprotective, and lipid-regulating properties—the presence and compositional richness of fatty acids in *C. kirikkaleensis* may indicate its potential as a supplementary source of medicinally valuable lipids (Djuricic & Calder, 2021; Mercola & D'Adamo, 2023). On the other hand, hexacosanoic acid (cerotic acid), which was detected in considerable amounts in the root extract, may serve as a valuable candidate for further investigations into certain cancer types and metabolic disorders (Kaufmann et al., 2023). The lipid composition of *C. medium* has been previously investigated in the literature. A total of 11 fatty acids were identified,

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with linoleic acid (C18:2) detected at the highest proportion, followed by oleic acid (C18:1). These two fatty acids accounted for approximately 83.1% of the total fatty acid methyl esters (FAME). Palmitic acid (C16:0) was identified as the predominant saturated fatty acid, constituting 10.8% of the total FAME, followed by stearic acid (C18:0). Long-chain fatty acids, such as lignoceric acid (C24:0) and eicosapentaenoic acid (EPA, C20:5), were found in trace amounts (Assiri et al., 2014). The presence of common fatty acids such as linoleic, palmitic, and stearic acids in both *C. kirikkaleensis* and *C. medium* highlights the shared fatty acid profile between these two species.

Studies on vitamins in *Campanula* species have reported the presence of carotenoids in the flowers of *C. alliariifolia*, *C. rapunculoides*, *C. latifolia*, and *C. persicifolia*. Additionally, ascorbic acid has been detected in the flowers of *C. alliariifolia*, *C. carpatica*, *C. latifolia*, *C. persicifolia*, *C. rapunculoides*, *C. sarmatica*, and *C. trachelium* (Fomina & Kukushkina, 2021). On the other hand, despite its diverse lipid composition, *C. kirikkaleensis* was found to lack detectable levels of fat-soluble vitamins—a distinction from other *Campanula* species (Fomina & Kukushkina, 2021). This absence might be attributed to species-specific metabolic characteristics, environmental factors influencing biosynthesis, or possible degradation during sample processing. Further comparative analyses are warranted to elucidate whether this feature is unique to *C. kirikkaleensis* or reflects a broader chemotaxonomic trait within the genus.

Furthermore, elemental analysis of the edible parts of *C. pelviformis* revealed the presence of aluminum (Al), boron (B), barium (Ba), beryllium (Be), calcium (Ca), cadmium (Cd), cobalt (Co), chromium (Cr), copper (Cu), iron (Fe), potassium (K), magnesium (Mg), manganese (Mn), sodium (Na), nickel (Ni), phosphorus (P), lead (Pb), silicon (Si), and zinc (Zn) (Tsiftsoglou et al., 2023). The absence of fat-soluble vitamins in *C. kirikkaleensis*, unlike some other *Campanula* species, may reflect species-specific metabolic traits and environmental influences, whereas the presence of common elements such as calcium and potassium suggests conserved physiological roles across these taxa. Calcium, though found in small concentrations in body fluids, functions as an intracellular signal and is vital for the normal operation of the heart, muscles, and nervous system, with the majority (~99%) deposited in bones and teeth as hydroxyapatite. Potassium is essential for sustaining proper mineral balance within the body (Tsiftsoglou et al., 2023).

The ability of medicinal plants to mitigate oxidative stress is a well-recognized indicator of their therapeutic potential (Syraji et al., 2024). In this context, *C. kirikkaleensis* demonstrated consistent antioxidant activity across multiple cell-free assays. The 70% methanol extract of the aerial part exhibited notable radical scavenging (DPPH) and electron-donating (CUPRAC) capacities, reflecting the presence of polar antioxidant constituents. Similarly, the chloroform extract of the root showed marked activity in the TEAC assay, highlighting the contribution of moderately polar compounds. These results point to the chemical diversity of *C. kirikkaleensis* and the relevance of solvent polarity in extracting its bioactive components. The antioxidant profile of *C. kirikkaleensis* also aligns with previous reports from related *Campanula* species (Jaradat & Abualhasan, 2015), indicating a potentially shared phytochemical defense mechanism within the genus. Considering the role of oxidative stress in various chronic diseases, including cardiovascular, metabolic, and neurodegenerative disorders, the observed antioxidant effects support the potential therapeutic value of this endemic species (Dumanović et al., 2021).

In the DPPH assay, both the root and aerial part methanol extracts exhibited stronger radical scavenging activity compared to the ethanol extract of *C. lyrata* leaves collected during the consumable period (IC_{50}: 1.07 ± 0.01 mg/mL) (Taşkın & Bitiş, 2016). Although the aerial part n-butanol extract of *C. alata* showed an IC_{50} of 25.77 ± 0.2 µg/mL, this value cannot be directly compared with the IC_{50} values reported in the present study, which are expressed in mg/mL. Therefore, only a qualitative comparison is possible. (Touafek et al., 2011). Although *C. lyrata* leaves were reported to exhibit 1.83 ± 0.006 mM Trolox/mg extract, this value is not directly comparable to the TEAC values reported in the present study due to differences in unit normalization. (Taşkın & Bitiş, 2016). The methanol (375.1 ± 12.2 mM TE) and chloroform (237.6 ± 3.1 mM TE) extracts of *C. kirikkaleensis* exhibited strong CUPRAC activity. Although the ethanolic extract of *C. glomerata* showed 99.89 ± 3.06 mg TE/g extract, these values are not directly comparable due to differences in reporting units. (Sarikurkcu et al., 2017). These results highlight that the antioxidant potential of *C. kirikkaleensis* varies depending on the type of extract and the analytical method employed, and suggest that specific fractions of the plant may offer valuable antioxidant properties for further pharmacological exploration.

The potential medicinal relevance and antioxidant effects of *C. kirikkaleensis* may be attributed to its diverse phenolic constituents. Phenolic compounds are increasingly recognized for their multifaceted roles in human health, owing to their antioxidant, anti-inflammatory, and disease-modulating properties that intersect with key pathways in chronic disease prevention and cellular defense (Lin et al., 2016; Luthar et al., 2020). Rutin—also known as vitamin P or rutoside—was detected at over 100-fold higher concentrations in the aerial part compared to the root of *C. kirikkaleensis*. The predominance of rutin in the aerial parts may reflect an adaptive strategy to protect photosynthetically active tissues against UV-induced oxidative stress, as rutin is known for its potent radical scavenging and metal-chelating properties (Suzuki et al., 2015). This flavonoid is recognized for its diverse pharmacological effects, including antithrombotic, antihypertensive, anti-inflammatory, antibacterial, antifungal, antimicrobial, and anticarcinogenic activities (Salkić et al., 2023). In contrast, the dominance of chlorogenic acid in the roots may be associated with defense against soil-borne pathogens and regulation of root metabolism (Niggeweg et al., 2004), while this compound has also demonstrated protective effects against cardiovascular disease and diabetes, primarily through its capacity to regulate inflammatory mediator release and exert potent

anti-inflammatory actions (Huang et al., 2023). Similarly, syringic acid, vanillic acid, and *p*-coumaric acid—also identified in *C. kirikkaleensis*—have been reported to exhibit comparable antioxidant and anti-inflammatory activities, further contributing to the therapeutic potential of this plant (Shimsa et al., 2023; Osorio-Paz et al., 2023; Chen et al., 2024). This correlation between the phenolic profile and assay outcomes indicates that the strong radical scavenging, reducing power, and enzyme inhibition observed in the methanolic aerial part extract are likely associated with its high rutin content, whereas the notable TEAC activity in the root extracts may be linked to the presence of chlorogenic acid and related phenolics. In previous studies, chlorogenic acid, hesperidin, and hyperoside were identified as the major components in the methanol extract of the aerial parts of *C. macrostachya* (Sarikurkcu et al., 2021). Moreover, chlorogenic acid was detected in the aerial parts of *C. persicifolia* at a concentration of 54.30 mg/100 g of dry plant material (Mogoşanu et al., 2019). In comparison with these findings, the aerial part extract of *C. kirikkaleensis* appears to contain a relatively higher amount of chlorogenic acid, indicating its potential as a valuable source of phenolic compounds.

Given that diabetes mellitus (DM) remains one of the most pressing global health challenges, evaluating the antidiabetic potential of medicinal plants has become a critical step in identifying novel therapeutic candidates (Telagari & Hullatti, 2015; Kashtoh & Baek, 2023). Based on the IC_{50} values, *C. kirikkaleensis*—particularly its 70% methanol extract of the aerial part—exhibited notable α-amylase inhibitory activity (0.54 ± 0.04 mg/mL), suggesting a moderate antidiabetic potential comparable to that of standard acarbose (0.22 ± 0.01 mg/mL). This highlights *C. kirikkaleensis* as a promising natural source of enzyme inhibitors relevant to glycemic control strategies (Méril-Mamert et al., 2022). In comparison with other *Campanula* species, *C. baskilensis* extracts exhibited stronger α-amylase inhibition, with IC_{50} values lower than that of acarbose (Marah, 2021). Although *C. glomerata* extract exhibited 0.54 ± 0.01 mmol acarbose equivalents/g extract (Sarikurkcu et al., 2017), this metric differs from IC_{50} values expressed in mg/mL; therefore, the two results cannot be directly compared. These findings suggest that although *C. kirikkaleensis* extracts exhibit α-amylase inhibitory activity, their potency is lower than that of *C. baskilensis* but similar to *C. glomerata*, indicating a moderate antidiabetic potential that merits further phytochemical investigation.

5 Limitations

This study has several limitations. First, all biological evaluations were conducted using cell-free *in-vitro* assays, and thus the *in-vivo* relevance of the findings remains to be validated. Second, the study focused on selected solvent fractions and a limited set of reference standards, which may not fully capture the complete metabolic or therapeutic potential of the species. Additionally, environmental factors such as harvest season and geography, which may influence phytochemical profiles, were not evaluated. Finally, the non-detection of certain compounds—such as fat-soluble vitamins—may reflect methodological sensitivity limitations rather than true absence.

6 Conclusions

This study represents the first comprehensive phytochemical profiling and cell-free bioactivity assessment of *C. kirikkaleensis*, an endemic species of Türkiye. The investigation encompassed essential oils, volatile constituents, fixed fatty acids, and phenolic profiles, alongside evaluations of antioxidant and antidiabetic activities. Notably, the aerial part oil was rich in phytol and long-chain hydrocarbons, while the root oil exhibited high levels of hexadecanoic acid and quinoxaline derivatives. Among the phenolics, rutin and chlorogenic acid were the dominant constituents, potentially contributing to the plant's strong cell-free antioxidant properties. Furthermore, α-amylase inhibition assays revealed promising antidiabetic potential, particularly in the 70% methanol extract of the aerial part. Overall, the findings highlight the pharmacological potential of *C. kirikkaleensis* and underscore the need for further studies—especially in vivo and mechanistic investigations—to validate and expand its therapeutic relevance.

Supplementary Materials

Table S1: Chemical composition of the essential oils from the aerial part and root of *C. kirikkaleensis* analyzed by GC-FID/MS, Table S2: GC-FID/MS analysis of the volatile compounds in *C. kirikkaleensis* aerial part and root using the MSD-SPME technique, Table S3: GC-FID/MS analysis of TMS derivatives of *C. kirikkaleensis* aerial part different extracts, Table S4: GC-FID/MS analysis of TMS derivatives of *C. kirikkaleensis* different root extracts.

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Author Contributions

Conceptualization, T.Ö. and M.Ç.; methodology, M.Ç., G.Ö. and T.Ö.; software, M.Ç.; validation, M.Ç. and T.Ö.; formal analysis, M.Ç., G.Ö, SY and T.Ö.; investigation, M.Ç.; resources, Ö.K.Y. and T.Ö.; data curation, M.Ç., G.Ö. and

T.Ö.; writing—original draft preparation, M.Ç.; writing—review and editing, M.Ç., G.Ö. and T.Ö.; visualization, M.Ç., Ö.K.Y., and T.Ö.; supervision, T.Ö.; project administration, T.Ö. and M.Ç.; funding acquisition, M.Ç., Ö.K.Y., and T.Ö. All authors have read and agreed to the published version of the manuscript.

Data Availability Statement

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Conflicts of Interest

The authors declare no conflicts of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Supporting Information

Supporting information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products.

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References

Adams, R. (2005). *Identification of Essential Oil Components by Gas Chromatography/Quadrupole Mass Spectroscopy, 4*, vol. *16*, 1st ed., pp. 65–120. Carol Stream, Allured Publishing. ISBN 978-1-932633-21-4.

Ahmed, E. A., Mohamed, M. F. A. & Omran, O. A. (2022). Novel quinoxaline derivatives as dual EGFR and COX-2 inhibitors: Synthesis, molecular docking and biological evaluation as potential anticancer and anti-inflammatory agents. *RSC Advances*, *12*(39), 25204–25216. DOI: 10.1039/d2ra04498f.

Ambarlı, D., Zeydanlı, U. S., Balkız, Ö., Aslan, S., Karaçetin, E., Sözen, M., Ilgaz, Ç., Gürsoy Ergen, A., Lise, Y., Demirbaş Çağlayan, S., Welch, H. J., Welch, G., Turak, A. S., Bilgin, C. C., Özkil, A. & Vural, M. (2016). An overview of biodiversity and conservation status of steppes of the Anatolian Biogeographical Region. *Biodivers Conserv*, *25*(12), 2491–2519. DOI: 10.1007/s10531-016-1172-0.

Aparna, V., Dileep, K. V., Mandal, P. K., Karthe, P., Sadasivan, C. & Haridas, M. (2012). Anti-inflammatory property of n-hexadecanoic acid: structural evidence and kinetic assessment. *Chemical Biology & Drug Design*, *80*(3), 434–439. DOI: 10.1111/j.1747-0285.2012.01418.x.

Assiri, A. M. A., Ramadan, M. F., Alshmy, H. & Morsel, J. T. (2014). Bioactive compounds and antiradical potential of *Campanula medium* lipids. *Chemistry of Natural Compounds*, *50*, 1088–1091. DOI: 10.1007/s10600-014-1165-8.

Aziz, M., Ahmad, S., Khurshid, U., Pervaiz, I., Lodhi, A. H., Jan, N., Khurshid, S., Arshad, M. A., Ibrahim, M. M., Mersal, G. A. M., Alenazi, F. S., Awadh Saleh Alamri, A., Butt, J., Saleem, H. & El-Bahy, Z. M. (2022). Comprehensive biological potential, phytochemical profiling using GC-MS and LC-ESI-MS, and in-silico assessment of *Strobilanthes glutinosus* Nees: An important medicinal plant. *Molecules*, *27*(20), 6885. DOI: 10.3390/molecules27206885.

Başer, K. H. C. & Demirci, F. (2007). Chemistry of essential oils. In: Berger, R. G. (Ed.), *Flavours and Fragrances* (pp. 43–86). Berlin, Heidelberg: Springer. 10.1007/978-3-540-49339-6_4.

Benali, T., Bakrim, S., Ghchime, R., Benkhaira, N., El Omari, N., Balahbib, A., Taha, D., Zengin, G., Hasan, M. M., Bibi, S. & Bouyahya, A. (2022). Pharmacological insights into the multifaceted biological properties of quinic acid. *Biotechnology and Genetic Engineering Reviews*, *40*(4), 3408–3437. DOI: 10.1080/02648725.2022.2122303.

Benramdane, E., Chougui, N., Ramos, P. A. B., Makhloufi, N., Tamendjari, A., Silvestre, A. J. D. & Santos, S. A. O. (2022). Lipophilic compounds and antibacterial activity of *Opuntia ficus-indica* root extracts from algeria. *International Journal of Molecular Sciences*, *23*(19), 11161. DOI: 10.3390/ijms231911161.

Bezmialem Vakıf University. ILMER Spectral Library—Chromatography and Spectroscopy Laboratory. Available online: https://bezmialem.edu.tr/ilacarastirma/en/Pages/facilities-and-analyses/facilities-and-analyses/chromatography-spectroscopy-laboratory.aspx. Accessed September 2024.

Bussmann, R. W., Paniagua Zambrana, N. Y., Sikharulidze, S., Kikvidze, Z., Kikodze, D., Tchelidze, D., Khutsishvili, M., Batsatsashvili, K. & Hart, R. E. (2016). A comparative ethnobotany of Khevsureti, Samtskhe-Javakheti, Tusheti, Svaneti, and Racha-Lechkhumi, Republic of Georgia (Sakartvelo). *Journal of Ethnobiology and Ethnomedicine*, *12*(1), 43. DOI: 10.1186/s13002-016-0110-2.

Çetin, M. (2025). *Phytochemical and Biological Activity Investigation of Endemic Campanula kirikkaleensis Dönmez & Güner* [Ph.D. thesis] Anadolu University, Graduate School of Health Sciences, Department of Pharmacognosy, Eskişehir, Turkey.

Chen, F., Zhang, X., Wang, J., Wang, F. & Mao, J. (2024). P-coumaric acid: Advances in pharmacological research based on oxidative stress. *Current Topics in Medicinal Chemistry*, *24*(5), 416–436. DOI: 10.2174/0115680266276823231230183519.

De Groot, A. C. & Schmidt, E. (2016a). Essential oils, Part III: Chemical composition. *Dermatitis*, *27*(4), 161–169. DOI: 10.1097/DER.0000000000000193.

De Groot, A. C. & Schmidt, E. (2016b). Tea tree oil: contact allergy and chemical composition. *Contact Dermatitis*, *75*(3), 129–143. DOI: 10.1111/cod.12591.

Dinicola, S., Unfer, V., Facchinetti, F., Soulage, C. O., Greene, N. D., Bizzarri, M., Laganà, A. S., Chan, S-Y., Bevilacqua, A., Pkhaladze, L., Benvenga, S., Stringaro, A., Barbaro, D., Appetecchia, M., Aragona, C., Bezerra Espinola, M. S., Cantelmi, T., Cavalli, P., Chiu, T. T., Copp, A. J. et al. (2021). Inositols: From established knowledge to novel approaches. *International Journal of Molecular Sciences*, *22*(19), 10575. DOI: 10.3390/ijms221910575.

Djuricic, I. & Calder, P. C. (2021). Beneficial outcomes of Omega-6 and Omega-3 polyunsaturated fatty acids on

human health: An update for 2021. *Nutrients*, *13*(7), 2421. DOI: 10.3390/nu13072421.

Dönmez, A. & Güner, A. (1993). A new species from Central Anatolia: *Campanula kirikkaleensis* (Campanulaceae). *The Karaca Arboretum Magazine*, *2*(3), 115–119.

Dumanović, J., Nepovimova, E., Natić, M., Kuča, K. & Jaćević, V. (2021). The significance of reactive oxygen species and antioxidant defense system in plants: A concise overview. *Frontiers in Plant Science*, *11*, 552969. DOI: 10.3389/fpls.2020.552969.

Erdoğan, A., Sarı, A. & Ozsoy, N. (2025). Phytochemical properties and antioxidant capacities of some *Campanula* species from Turkey. *Spectroscopy Letters*, *58*(8), 1–12. DOI: 10.1080/00387010.2025.2467430.

European Directorate for the Quality of Medicines & HealthCare. (2023). Essential oils (Monograph 2098). In *European Pharmacopoeia* (11th ed.). Strasbourg: Council of Europe. https://www.edqm.eu/en/european-pharmacopoeia-ph.-eur.-11th-edition

Fomina, T. & Kukushkina, T. (2021). Flowers of *Campanula* species as a source of biologically active substances. *BIO Web of Conferences*, *38*, 00033. DOI: 10.1051/bioconf/20213800033.

Gibbs, J. E. (2019). Essential oils, asthma, thunderstorms, and plant gases: a prospective study of respiratory response to ambient biogenic volatile organic compounds (BVOCs). *Journal of Asthma and Allergy*, *12*, 169–182. DOI: 10.2147/JAA.S193211.

Huang, J., Xie, M., He, L., Song, X. & Cao, T. (2023). Chlorogenic acid: A review on its mechanisms of anti-inflammation, disease treatment, and related delivery systems. *Frontiers in Pharmacology*, *14*, 1218015. DOI: 10.3389/fphar.2023.1218015.

Islam, M. T., Ali, E. S., Uddin, S. J., Shaw, S., Islam, M. A., Ahmed, M. I., Chandra Shill, M., Karmakar, U. K., Yarla, N. S., Khan, I. N., Billah, M. M., Pieczynska, M. D., Zengin, G., Malainer, C., Nicoletti, F., Gulei, D., Berindan-Neagoe, I., Apostolov, A., Banach, M. & Yeung, A. W. K. (2018). Phytol: A review of biomedical activities. *Food and Chemical Toxicology*, *121*(6), 82–94. DOI: 10.1016/j.fct.2018.08.032.

Jaradat, N. A. & Abualhasan, M. (2015). Comparison in vitro of antioxidant activity between Fifteen *Campanula* species (Bellflower) from Palestinian Flora. *Pharmacognosy Journal*, *7*(5), 276–279. DOI: 10.5530/pj.2015.5.4.

Joulain, D., König, W. A. & Hochmuth, D. H. (2001). Terpenoids and related constituents of essential oils. *Acta Pharmaceutica*, *57*, 90–123.

Kashima, H., Honma, A., Kamimura, S., Nishimura, S., Sano, T., Matsumoto, S., Endo, M. Y. & Fukuba, Y. (2021). Heated corn oil and 2,4-decadienal suppress gastric emptying and energy intake in humans. *Nutrients*, *13*(4), 1304. DOI: 10.3390/nu13041304.

Kashtoh, H. & Baek, K.-H. (2023). New insights into the latest advancement in α-amylase inhibitors of plant origin with anti-diabetic effects. *Plants*, *12*(16), 2944. DOI: 10.3390/plants12162944.

Kaufmann, M., Iaboni, N., Jamzad, A., Hurlbut, D., Ren, K. Y. M., Rudan, J. F., Mousavi, P., Fichtinger, G., Varma, S., Caycedo-Marulanda, A. & Nicol, C. J. B. (2023). Metabolically active zones involving fatty acid elongation delineated by DESI-MSI correlate with pathological and prognostic features of colorectal cancer. *Metabolites*, *13*(4), 508. DOI: 10.3390/metabo13040508.

Kim, M.-S., Kim, K.-H. & Yook, H.-S. (2012). Antioxidative effects of *Campanula takesimana* Nakai extract. *Journal of the Korean Society of Food Science and Nutrition*, *41*(10), 1331–1337. DOI: 10.3746/jkfn.2012.41.10.1331.

Korkmaz, B., Fandaklı, S., Barut, B., Yıldırım, S., Sener, S. O., Ozturk, E., Terzioğlu, S. & Yayli, N. (2020). Volatile and phenolic components and antioxidant, acetylcholinesterase, tyrosinase, α-glucosidase inhibitory effects of extracts obtained from *Campanula latifolia* L. subsp. latifolia. *Journal of Essential Oil Bearing Plants*, *23*(5), 1118–1131. DOI: 10.1080/0972060X.2020.1856004.

Krishnaiah, D., Sarbatly, R. & Nithyanandam, R. (2011). A review of the antioxidant potential of medicinal plant species. *Food and Bioproducts Processing*, *89*(3), 217–233. DOI: 10.1016/j.fbp.2010.04.008.

Kyoui, D., Saito, Y., Takahashi, A., Tanaka, G., Yoshida, R., Maegaki, Y., Kawarai, T., Ogihara, H. & Suzuki, C. (2023). Antibacterial activity of hexanol vapor in vitro and on the surface of vegetables. *Foods*, *12*(16), 3097. DOI: 10.3390/foods12163097.

Lin, D., Xiao, M., Zhao, J., Li, Z., Xing, B., Li, X., Kong, M., Li, L., Zhang, Q., Liu, Y., Chen, H., Qin, W., Wu, H. & Chen, S. (2016). An overview of plant phenolic compounds and their importance in human nutrition and management of Type 2 diabetes. *Molecules*, *21*(10), 1374. DOI: 10.3390/molecules21101374.

Liu, Z., Liu, H., Chen, Z., Deng, C., Zhou, L., Chen, S., Kang, J., Chen, Y., He, S. & Zhou, Z. (2023). Identification of a novel plasma metabolite panel as diagnostic biomarker for hepatocellular carcinoma. *Clinica Chimica Acta*, *543*(2), 117302. DOI: 10.1016/j.cca.2023.117302.

Liveri, E., Crowl, A. A. & Cellinese, N. (2019). Past, present, and future of *Campanula* (Campanulaceae) systematics—A review. *Botanika Chronika Patras*, *22*, 209–222.

Luthar, Z., Germ, M., Likar, M., Golob, A., Vogel-Mikuš, K., Pongrac, P., Kušar, A., Pravst, I. & Kreft, I. (2020). Breeding buckwheat for increased levels of rutin, quercetin and other bioactive compounds with potential antiviral effects. *Plants*, *9*(12), 1638. DOI: 10.3390/plants9121638.

Marah, H. S. M. (2021). *Phytochemical and bioactivity studies of the endemic plant Campanula baskilensis Behçet (Campanulaceae)* [Master's thesis]. Ondokuz Mayıs University, Graduate School of Education.

Marah, S., Ozen, T., Demirtas, İ., İpek, Y., Gül, F. & Behçet, L. (2024). GC-MS/MS and 1H-NMR analysis of endemic *Campanula baskilensis* Behçet leaf fractions. *Current Perspectives on Medicinal and Aromatic Plants (CUPMAP)*, *7*(1), 48–57. DOI: 10.38093/cupmap.1477900.

Marrero Delange, D. & González Bravo, L. (2001). Trace determination of 1-octacosanol in rat plasma by solid-phase extraction with tenax GC and capillary gas chromatography. *Journal of Chromatography B: Biomedical Sciences and Applications*, *762*(1), 43–49. DOI: 10.1016/s0378-4347(01)00331-0.

Mathe, E., Sethoga, L., Mapfumari, S., Adeniran, O., Mokgotho, P., Shai, J. & Gololo, S. (2024). Phytochemical screening and characterization of volatile compounds from three medicinal plants with reported anticancer properties using GC-MS. *Life (Basel)*, *14*(11), 1375. DOI: 10.3390/life14111375.

Mercola, J. & D'Adamo, C. R. (2023). Linoleic acid: A narrative review of the effects of increased intake in the standard american diet and associations with chronic disease. *Nutrients*, *15*(14), 3129. DOI: 10.3390/nu15143129.

Méril-Mamert, V., Ponce-Mora, A., Sylvestre, M., Lawrence, G., Bejarano, E. & Cebrián-Torrejón, G. (2022). Antidiabetic potential of plants from the caribbean basin. *Plants*, *11*(10), 1360. DOI: 10.3390/plants11101360.

Mogoşanu, G. D., Bejenaru, C., Bejenaru, L. E., Biţă, A., Blendea, A. & Scorei, I. D. (2019). Histo-anatomical and chromatographic researches on *Campanula persicifolia* L. (*Campanulaceae*) species. *Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series*, *48*, 95–104.

Mok, B., Jang, Y. S., Moon, J. H., Moon, S., Jang, Y. K., Kim, S. Y., Jang, S. J., Moh, S. H., Kim, D. H. & Shin, J. U. (2023). The potential of *Campanula takesimana* callus extract to enhance skin barrier function. *International Journal of Molecular Sciences*, *24*(24), 17333. DOI: 10.3390/ijms242417333.

Möller, R., Nürnberg, G., Albrecht, E., Ruth, W., Brockmann, G. & Dannenberger, D. (2019). A method for analyzing fatty acids in cattle hair, with special emphasis on lauric acid and myristic acid. *European Journal of Lipid Science and Technology*, *121*(11), 121–111. DOI: 10.1002/ejlt.201900143.

Niggeweg, R., Michael, A. J. & Martin, C. (2004). Engineering plants with increased levels of the antioxidant chlorogenic acid. *Nature Biotechnology*, *22*(6), 746–754. DOI: 10.1038/nbt966.

Nithish, S., Anvesh, M., Sanjana, A. R., Rani, R. U., Pranay, R. & Vydhi, A. (2021). An in vitro study of effect of salt and sugar on bacterial species. *International Journal of Current Microbiology and Applied Sciences*, *10*(2), 1034–1042. DOI: 10.20546/ijcmas.2021.1002.122.

Osorio-Paz, I., Valle-Jiménez, X., Brunauer, R. & Alavez, S. (2023). Vanillic acid improves stress resistance and substantially extends life span in *Caenorhabditis elegans*. *The Journals of Gerontology: Series A*, *78*(7), 1100–1107. DOI: 10.1093/gerona/glad086.

Ozcan, M. & Eminagaoglu, O. (2018). Anatomical characteristics and conservation status of endemic *Campanula betulifolia* and *C. choruhensis* (Campanulaceae). *Turkish Journal of Biodiversity*, *1*(1), 11–16.

Ozek, G., Ozek, T., Iscan, G., Baser, K. H. C., Hamzaoğlu, E. & Duran, A. (2007). Comparison of hydrodistillation and microdistillation methods for the analysis of fruit volatiles of *Prangos pabularia* Lindl., and evaluation of its antimicrobial activity. *South African Journal of Botany*, *73*(4), 563–569. DOI: 10.1016/j.sajb.2007.05.002.

Özek, G., Shukurlu, E. N., Özek, T., Bertolini, C., Iriti, M., Pezzani, R. & Vitalini, S. (2025). A comparative MSD-SPME-GC/MS analysis of two *Artemisia* species from the Rhaetian alps (Italy). *Natural Product Research*, Ahead of Print, 1–5. DOI: 10.1080/14786419.2025.2491116.

Özek, G., Özek, T., Başer, K., Duran, A., Sagiroglu, M. & Duman, H. (2006). Comparison of the essential oils of *Prangos turcica* A. Duran, M. Sagiroglu et H. Duman fruits obtained by different isolation techniques. *Journal of Essential Oil Research*, *18*(5), 511–514. DOI: 10.1080/10412905.2006.9699156.

Özyürek, M., Güçlü, K. & Apak, R. (2011). The main and modified CUPRAC methods of antioxidant measurement. *Trends in Analytical Chemistry*, *30*(4), 652–664. DOI: 10.1016/j.trac.2010.11.016.

Pezantes-Orellana, C., Bermúdez, F. G., De la Cruz, C. M., Montalvo, J. L. & Orellana-Manzano, A. (2024). Essential oils: a systematic review on revolutionizing health, nutrition, and omics for optimal well-being. *Frontiers in Medicine (Lausanne)*, *11*, 1337785. DOI: 10.3389/fmed.2024.1337785.

Pironon, S., Ondo, I., Diazgranados, M., Allkin, R., Baquero, A. C., Cámara-Leret, R., Canteiro, C., Dennehy-Carr, Z., Govaerts, R., Hargreaves, S., Hudson, A. J., Lemmens, R., Milliken, W., Nesbitt, M., Patmore, K., Schmelzer, G., Turner, R. M., van Andel, T. R., Ulian, T. & Antonelli, A. (2024). The global distribution of plants used by humans. *Science*, *383*(6680), 293–297. DOI: 10.1126/science.adg8028.

Pniewska, A. & Kalinowska-Lis, U. (2024). A survey of UV filters used in sunscreen cosmetics. *Applied Sciences*, *14*(8), 3302. DOI: 10.3390/app14083302.

Proestos, C. & Komaitis, M. (2013). Analysis of naturally occurring phenolic compounds in aromatic plants by RP-HPLC coupled to diode array detector (DAD) and GC-MS after silylation. *Foods*, *2*(1), 90–99. DOI: 10.3390/foods2010090.

Ramli, Y., Moussaif, A., Karrouchi, K. & Essassi, E. M. (2014). Pharmacological profile of quinoxalinone. *Journal of Chemistry*, *2014*(11), 563406, 1–21. DOI: 10.1155/2014/563406.

Ramsey, J. T., Shropshire, B. C., Nagy, T. R., Chambers, K. D., Li, Y. & Korach, K. S. (2020). Essential oils and health. *Yale Journal of Biology and Medicine*, *93*(2), 291–305. PMID: 32607090; PMCID: PMC7309671.

Razboršek, M., Vončina, D., Doleček, V. & Voncina, E. (2008). Determination of oleanolic, betulinic and ursolic acid in Lamiaceae and mass spectral fragmentation of their trimethylsilylated derivatives. *Chromatographia*, *67*(5-6), 433–440. DOI: 10.1365/s10337-008-0533-6.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, *26*(9-10), 1231–1237. DOI: 10.1016/s0891-5849(98)00315-3.

Salajeghe, M., Moradalizadeh, M., Mehrabpanah, M. & Heidari, A. (2015). Chemical constituents of the essential oil of *Campanula kermanica* from Kerman, Iran. *Chemistry of Natural Compounds*, *51*(2), 361–362. DOI: 10.1007/s10600-015-1284-x.

Salkić, A., Mujezin, L., Džafić, A., Kučuk, Z. B. & Žuljević, S. O. (2023). The importance of rutin in nutrition. *Proceedings*, *91*, 236. DOI: 10.3390/proceedings2023091236.

Sarikurkcu, C., Sarikurkcu, R. T. & Tepe, B. (2021). *Campanula macrostachya*: Biological activity and identification of phenolics using a liquid chromatography electrospray ionization tandem mass spectrometry system. *Environmental Science and Pollution Research*, *28*(17), 21812–21822. DOI: 10.1007/s11356-020-11695-y.

Sarikurkcu, C., Targan, S., Ozer, M. S. & Tepe, B. (2017). Fatty acid composition, enzyme inhibitory, and antioxidant activities of the ethanol extracts of selected wild edible plants consumed as vegetables in the Aegean region of Turkey. *International Journal of Food Properties*, *20*(3), 560–572. DOI: 10.1080/10942912.2016.1168837.

Şenkul, Ç. & Kaya, S. (2017). Geographical distribution of endemic plants of Turkey. *Türk Coğrafya Dergisi*, (69), 109–120. DOI: 10.17211/tcd.322515.

Shimsa, S., Soumya, N., Mondal, S. & Saraswathy, M. (2023). Syringic acid affords antioxidant protection in the pancreas of Type 2 diabetic rats. *Bioactive Compounds in Health and Disease*, *6*(2), 13. DOI: 10.31989/bchd.v6i2.1061.

Sinek, K., Iskender, N. Y., Yayli, B., Karaoglu, S. A. & Yayli, N. (2012). Antimicrobial activity and chemical composition of the essential oil from *Campanula glomerata* L. subsp. *hispida* (Witasek) Hayek. *Asian Journal of Chemistry*, *24*, 1931–1934.

Suzuki, T., Morishita, T., Kim, S. J., Park, S. U., Noda, T. & Takigawa, S. (2015). Physiological roles of rutin in the buckwheat plant. *Japan Agricultural Research Quarterly: JARQ*, *49*(1), 37–43. DOI: 10.6090/jarq.49.37.

Syraji, Y., Kebebew, M., Techane, Y. & Albene, D. (2024). Antimicrobial, antiradical activity, and X-ray fluorescence spectroscopy analysis of aloe otallensis plant used in traditional medicine in Southern Ethiopia. *International Journal of Microbiology*, *2024*, 1981990. DOI: 10.1155/2024/1981990.

Taşkın, T. & Bitiş, L. (2016). *In vitro* antioxidant activity of eight wild edible plants in Bursa province of Turkey. *Farmacia*, *64*, 706–711.

Telagari, M. & Hullatti, K. (2015). In-vitro α-amylase and α-glucosidase inhibitory activity of *Adiantum caudatum* Linn. and *Celosia argentea* Linn. extracts and fractions. *Indian Journal of Pharmacology*, *47*(4), 425–429. DOI: 10.4103/0253-7613.161270.

Tosun, G., Kahriman, N., Çoskunçelebi, K., Genc, H., Karaoglu, S. A. & Yayli, N. (2011). Chemical composition and biological activity of the essential oil of Campanula olympica Boiss. *Asian Journal of Chemistry*, *23*(6), 2389–2391. https://asianpubs.org/index.php/ajchem/article/view/10457. Accessed on: Dec 1, 2024.

Touafek, O., Kabouche, Z., Brouard, I. & Barrera Bermejo, J. (2011). Flavonoids of *Campanula alata* and their antioxidant activity. *Chemistry of Natural Compounds*, *46*(6), 968–970. DOI: 10.1007/s10600-011-9799-2.

Tsiftsoglou, O. S., Lagogiannis, G., Psaroudaki, A., Vantsioti, A., Mitić, M. N., Mrmošanin, J. M. & Lazari, D. (2023). Phytochemical analysis of the aerial parts of *Campanula pelviformis* Lam. (Campanulaceae): Documenting the dietary value of a local endemic plant of Crete (Greece) traditionally used as wild edible green. *Sustainability*, *15*(9), 7404. DOI: 10.3390/su15097404.

Urker, O. (2021). Up-to-date evaluation of the priority plant taxa and habitats of Kırıkkale province. *Bitlis Eren Üniversitesi Fen Bilimleri Dergisi*, *10*(1), 24–41. DOI: 10.17798/bitlisfen.817184.

Wang, H., Wang, Z., Zhang, Z., Liu, J. & Hong, L. (2023). β-Sitosterol as a promising anticancer agent for chemoprevention and chemotherapy: Mechanisms of action and future prospects. *Advances in Nutrition*, *14*(5), 1085–1110. DOI: 10.1016/j.advnut.2023.05.013.

Wiley Science Solutions. (2024). *GC-MS Spectral Databases*. John Wiley & Sons, Inc. Available online: https://sciencesolutions.wiley.com/solutions/technique/gc-ms. Accessed December 2024.

World Flora Online (WFO). (2025). *Campanula kirikkaleensis* Dönmez & Güner. Published on the Internet. http://www.worldfloraonline.org/taxon/wfo-0000827547. Accessed April 01, 2025.

Zavala, M., Rodríguez Chávez, J. L., Brito, R., Quintana-López, C., Bah, M., Campos-Guillén, J., Bustos Martinez, J., Zamora-Avella, D. & Ramos-Lopez, M. (2020). Bioactivity of 1-Octacosanol from *Senna crotalarioides* (Fabaceae: Caesalpinioideae) to control *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Florida Entomologist*, *102*(4), 731. DOI: 10.1653/024.102.0410.

Zengin, G., Aktumsek, A. & Ceylan, R. (2014). Sideritis galatica Bornm.: A source of multifunctional agents for the management of oxidative damage, Alzheimer's and diabetes mellitus. *Journal of Functional Foods*, *11*(10), 538–547. DOI: 10.1016/j.jff.2014.08.011.

Zhang, P., Liu, N., Xue, M., Zhang, M., Xiao, Z., Xu, C., Fan, Y., Qiu, J., Zhang, Q. & Zhou, Y. (2024). β-Sitosterol reduces the content of triglyceride and cholesterol in a high-fat diet-induced non-alcoholic fatty liver disease zebrafish (*Danio rerio*) model. *Animals*, *14*(9), 1289. DOI: 10.3390/ani14091289.

Zhou, Y., Cao, F., Luo, F. & Lin, Q. (2022). Octacosanol and health benefits: Biological functions and mechanisms of action. *Food Bioscience*, *47*(3), 101632. DOI: 10.1016/j.fbio.2022.101632.