Karadeniz Fen Bilimleri Dergisi, 15(3), 1196-1219, 2025. DOI: 10.31466/kfbd.1641998

KFBD

Karadeniz Fen Bilimleri Dergisi
The Black Sea Journal of Sciences
ISSN (Online): 2564-7377 https://dergipark.org.tr/tr/pub/kfbd

Araştırma Makalesi / Research Article

Phytochemical and Antioxidant Characterization of *Centaurea macrocephala* Willd.: With Notes on Chemotaxonomic Relevance

Nurşen AKSU KALMUK[1*]

Abstract

Centaurea macrocephala Willd. (Asteraceae) is a perennial herbaceous plant that has been relatively understudied in terms of its phytochemical composition and chemotaxonomic classification. Various *Centaurea* species are widely recognized for their medicinal properties. However, the phenolic composition, antioxidant activity, and volatile profile of *C. macrocephala* remain largely unexplored. This study aims to comprehensively evaluate the phytochemical profile and antioxidant properties of *C. macrocephala*, focusing on its flowers, leaves, and stems. The phenolic composition was determined using High-Performance Liquid Chromatography (HPLC), while antioxidant activity was assessed through Ferric Reducing Antioxidant Power (FRAP) and Copper Reducing Antioxidant Capacity (CUPRAC) assays. Additionally, volatile compounds were analyzed using Gas Chromatography-Mass Spectrometry coupled with Solid-Phase Microextraction (GC-MS-SPME). The leaves showed the highest levels of total phenolic and flavonoid compounds, which may explain the stronger antioxidant responses observed in FRAP and CUPRAC results. HPLC analysis identified chlorogenic acid, rutin, and quercetin derivatives as the dominant phenolic compounds. GC-MS-SPME analysis revealed that the volatile composition of *C. macrocephala* is predominantly characterized by aldehydes and ketones, distinguishing it from other *Centaurea* species, which are typically rich in sesquiterpenes.

Keywords: *Centaurea macrocephala*, Chemotaxonomy, Antioxidant activity, HPLC, GC-MS-SPME.

Centaurea *macrocephala* Willd.'in Fitokimyasal ve Antioksidan Karakterizasyonu: Kemotaksonomik Öneme Dair Notlarla

Öz

Centaurea macrocephala Willd. (Asteraceae), fitokimyasal bileşimi ve kemotaksonomik sınıflandırması açısından nispeten az çalışılmış, çok yıllık otsu bir bitkidir. Çeşitli *Centaurea* türleri tıbbi özellikleriyle geniş ölçüde tanınmaktadır. Ancak *C. macrocephala*'nın fenolik bileşimi, antioksidan aktivitesi ve uçucu bileşen profili büyük ölçüde araştırılmamıştır. Bu çalışma, *C. macrocephala*'nın çiçek, yaprak ve gövde kısımlarına odaklanarak fitokimyasal profilini ve antioksidan özelliklerini kapsamlı bir şekilde değerlendirmeyi amaçlamaktadır. Fenolik bileşim, Yüksek Performanslı Sıvı Kromatografi (HPLC) yöntemiyle belirlenirken, antioksidan aktivite ise Demir İyonu İndirgeme Antioksidan Gücü (FRAP) ve Bakır İyonu İndirgeme Kapasitesi (CUPRAC) analizleriyle ölçülmüştür. Ayrıca, uçucu bileşenler Katı Faz Mikroekstraksiyon ile Eşleştirilmiş Gaz Kromatografi-Kütle Spektrometrisi (GC-MS-SPME) yöntemi kullanılarak analiz edilmiştir. Yapraklar, toplam fenolik ve flavonoid bileşiklerin en yüksek düzeylerini göstermiştir; bu durum, FRAP ve CUPRAC sonuçlarında gözlemlenen daha güçlü antioksidan tepkileri açıklayabilir. HPLC analizi, klorojenik asit, rutin ve kuersetin türevlerinin baskın fenolik bileşikler olduğunu ortaya koymuştur. GC-MS-SPME analizi, *C. macrocephala*'nın uçucu bileşik profilinin esas olarak aldehitler ve ketonlarla karakterize edildiğini göstermiştir. Bu durum, tipik olarak sekiterpenler açısından zengin olan diğer *Centaurea* türlerinden farklılık göstermektedir.

Anahtar Kelimeler: *Centaurea macrocephala*, Kemotaksonomi, Antioksidan aktivite, HPLC, GC-MS-SPME.

[1]Artvin Çoruh University, Medicinal and Aromatic Plants Research and Application Center, Artvin, Türkiye, nrsnaksu@hotmail.com

[*]Sorumlu Yazar/Corresponding Author

Geliş/Received: 18.02.2025 **Kabul/Accepted:** 04.08.2025 **Yayın/Published:** 15.09.2025

Aksu Kalmuk, N. (2025). Phytochemical and Antioxidant Characterization of Centaurea macrocephala Willd.: With Notes on Chemotaxonomic Relevance. *Karadeniz Fen Bilimleri Dergisi*, 15(3), 1196-1219.

1. Introduction

Centaurea macrocephala Willd. (Asteraceae) is a perennial herbaceous plant belonging to the genus *Centaurea*, known for its striking yellow flower heads and high ornamental value. With approximately 600 species worldwide, 182 of which are found in Türkiye, this genus undescores the country's botanical diversity and ecological richness (Sarker et al., 2005; Özel and Maesaroh, 2023). *C. macrocephala* has erect stems reaching up to 150 cm and lanceolate to ovate leaves. Its natural distribution includes the Caucasus region—northeastern Türkiye, Armenia, and Iran—yet it has also been introduced to other countries and has established naturalized populations in some areas (Łazarski and Pliszko, 2022).

Many species of the genus *Centaurea* have been documented in folk medicine for their anti-inflammatory, antidiabetic, antimicrobial, and wound-healing uses. For example, *Centaurea calcitrapa* and *Centaurea iberica* have traditionally been used to reduce fever and alleviate inflammation caused by abscesses (Sezik et al., 2001; Shoeb et al., 2005). The absence of information regarding the use of *C. macrocephala* in traditional medicine suggests that this species has not yet been sufficiently studied from an ethnobotanical perspective. However, some phytochemical studies have reported the presence of lignan compounds in its seeds—such as arctiin and matairesinoside—which have drawn attention in the literature for their potential biological effects (Shoeb et al., 2007).

Existing studies aiming to reveal the phytochemical diversity of *Centaurea* species have generally focused on the analysis of phenolic compounds by high-performance liquid chromatography (HPLC) and volatile compounds by gas chromatography–mass spectrometry (GC-MS) (Sarker et al., 2005; Shoeb et al., 2007). However, these studies have mostly examined a single plant part—such as flowers or seeds—while other parts like stems and leaves are often neglected. Moreover, the use of solid-phase microextraction combined with GC-MS (SPME-GC-MS) in the analysis of volatile compounds in *Centaurea* species is quite limited. This method offers the advantage of extracting volatiles directly without the need for solvents or high temperatures. As a result, it provides a volatile profile that is more representative of the plant's natural emissions and more consistent with its ecological functions. (Pawliszyn, 1997; Zhang et al., 1994).

Previous chemotaxonomic studies have shown that flavonoid and phenolic acids such as chlorogenic acid, quercetin, and rutin can be important markers in distinguishing between species (Mishio et al., 2015; De Oliveira et al., 2017). The presence or absence of these compounds may reflect evolutionary divergence and ecological adaptations. For instance, while some species such as *Centaurea calolepis* are dominated by sesquiterpene lactones (e.g., cnicin), *C. macrocephala* has been found to have a volatile profile rich in aldehydes and ketones (Baykan Erel et al., 2011; Shoeb et al., 2007). The variation in volatile and phenolic compound composition across different *Centaurea*

species in response to ecological pressures—such as pollinator attraction or defense strategies—suggests that these chemical profiles are shaped by adaptive processes (Pichersky and Gershenzon, 2002). Furthermore, the presence of distinct metabolite patterns even among morphologically similar species indicates that these compounds may also reflect evolutionary processes (Wink, 2003).

In this study, the flowers, leaves, and stems of *Centaurea macrocephala* were individually analyzed to determine their total phenolic and flavonoid contents, antioxidant potential through FRAP and CUPRAC assays, phenolic composition via HPLC, and volatile compound profile using SPME-GC-MS. By integrating these complementary analytical approaches, a comprehensive and organ-specific understanding of the phytochemical structure of the species was developed. The study primarily aims to provide a detailed phytochemical profile of *C. macrocephala* across its different organs, to offer preliminary insights into its potential biological activities, and to contribute to chemotaxonomic assessments by enabling comparisons with related taxa based on the chemical data obtained.

2. Materials and Methods

2.1. Source of Plant Material

Figure 1 shows a habitat photograph of *C. macrocephala*, collected on July 5, 2024, from the Demirkent-Tanuz region of Yusufeli district, Artvin, at an altitude of 1900 meters in grassy areas. The plant was sampled both for experimental studies and for preparation as herbarium material. During sampling, habitat and population details were recorded, notes were taken regarding traits that might change after the plants were dried, and photographs were taken. The taxonomic identification and verification of the specimens were conducted based on the *Centaurea* taxonomic keys provided by Wagenitz (1975) and Ekim et al. (2012). Voucher specimens (Aksu 576) are preserved in the personal collection at the Artvin Çoruh University Medicinal and Aromatic Plants Application and Research Centre.

Figure 1. *Centaurea macrocephala* habit.

2.2. Preparation and Processing of Extracts

The plant samples were dried for 5 to 7 days, after which the flowers, leaves, and stems were separated and finely ground using a laboratory mill. Extraction was carried out by mixing each powdered sample with methanol and agitating the solution in a shaker at 25 °C for 24 hours. The resulting mixtures were then filtered through standard filter paper and concentrated using a rotary evaporator. The extracts were subsequently analyzed to determine their antioxidant properties, including total polyphenol and flavonoid content. Further evaluations included the Ferric Reducing Antioxidant Power (FRAP) and Copper (II) Reducing Antioxidant Capacity (CUPRAC) assays. Additionally, the chemical profiles of the extracts were examined using HPLC analysis.

2.3. Measurement of Total Phenolic Content

Total phenolic content was determined by allowing the sample to react with Folin-Ciocalteu reagent, which forms a blue-colored complex absorbing light at 760 nm. The depth of the color reflects the amount of phenolic compounds present in the sample. A standard calibration curve was prepared using gallic acid, allowing the phenolic content to be reported in gallic acid equivalents (GAE) (Singleton et al., 1999).

2.4. Measurement of Total Flavonoid Content

The total flavonoid content is determined using a modified version of the method outlined by Zhishen et al. (1999). This technique relies on the reaction of flavonoids with aluminum chloride, which forms stable complexes with the C-4 keto group and the C-3 or C-5 hydroxyl groups in flavones and flavonols. Additionally, it produces weaker complexes with ortho-dihydroxy groups located on the A and B rings. Quercetin serves as the standard, with concentrations ranging from 0.03125 to 1.0 mg/mL. A calibration curve is created by correlating absorbance values with these predefined concentrations.

2.5. Evaluation of Ferric Reducing Antioxidant Power (FRAP)

In an acidic environment, the antioxidants in the sample facilitate the reduction of the ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) to its ferrous form (Fe^{2+}-TPTZ), creating a blue-colored compound. The depth of the blue color, which signifies the reducing capacity of the antioxidants, is measured spectrophotometrically at 593 nm. The FRAP values of the methanolic extracts are reported as milligrams of quercetin equivalents per gram of sample, reflecting the extracts' ability to reduce ferric ions effectively (Benzie and Strain, 1996; Prior et al., 2005).

2.6. Evaluation of Copper Ion Reduction Assay (CUPRAC)

The CUPRAC method evaluates antioxidant capacity by monitoring the reduction of the copper(II)-neocuproine complex into its copper(I)-neocuproine form. This reaction, facilitated by antioxidants, produces a colored compound with a peak absorbance at 450 nm. For calibration, Trolox, a water-soluble derivative of vitamin E, was utilized as the standard at concentrations ranging from 0.03125 to 1 mM. The antioxidant capacity of the samples was quantified and expressed as

Trolox Equivalent Antioxidant Capacity (TEAC), providing a standardized measure of antioxidant potential relative to Trolox (Özyürek et al., 2011).

2.7. Analysis of Extracts by HPLC

To enable comprehensive phenolic profiling across structurally diverse compounds, two complementary HPLC methods were employed in this study. Both methods utilized the same ACE 5 C18 column (250 × 4.6 mm, i.d.) to ensure consistency in stationary phase selectivity.

Method 1 was primarily optimized for the detection of common phenolic acids and flavonols. Chromatographic separation was performed using a mobile phase composed of (A) acetonitrile and (B) 1.5% acetic acid solution. The gradient program began at 15% A and 85% B and was linearly adjusted to 40% A and 60% B over 29 minutes. The HPLC system included a 1260 DAD WR detector operating at 250, 270, and 320 nm, a 1260 Quaternary Pump with a flow rate of 0.7 mL/min, a 1260 Vialsampler injecting 10 µL of sample, and a G7116A column oven maintained at 35 °C.

Method 2 was designed to improve resolution for compounds with extended conjugated systems or alternative absorption maxima. It used a mobile phase of (A) methanol and (B) 1.5% acetic acid. The gradient was initiated at 10% A and 90% B, ramping to 40% A and 60% B at 29 minutes, followed by 60% A and 40% B from 29 to 40 minutes, and finally reaching 90% A and 10% B between 40 and 53 minutes. The detection wavelengths were adjusted to 280, 290, 320, 370, and 535 nm to cover a broader range of phenolic and flavonoid compounds. The system setup mirrored Method 1 in terms of flow rate, injection volume, and temperature settings.

This dual-method approach enabled more reliable quantification and characterization of the phenolic composition by accommodating the chemical diversity of targeted compounds.

2.8. Profiling Volatile Components via HS-SPME-GC-MS

To analyze volatile compounds, 3 g of dried samples from each plant part were placed into 20 mL glass vials and securely sealed with silicone/PTFE septa. The headspace-solid phase microextraction (HS-SPME) technique was employed, utilizing a fiber coated with an 80 µm layer of divinylbenzene/carbon wide range/polydimethylsiloxane (DVB/C-WR/PDMS) (PAL SYSTEM, Switzerland) in an automatic HS-SPME autosampler (PAL RSI, PAL System). Samples were incubated at 50 °C for 10 minutes before exposing the fiber to the headspace for an additional 10 minutes. The fiber was then manually inserted into the GC injector port for thermal desorption of the analytes, which was carried out for 10 minutes under splitless conditions. This approach enabled

direct transfer of the volatiles into the GC column without any intermediate solvent step, ensuring efficient and representative profiling of the plant's natural emissions.

A capillary column (HP-5MS, Agilent Technologies, Inc., 30 m × 0.25 mm, 0.25 μm) was employed, with helium as the carrier gas at a flow rate of 1 mL/min. The inlet was operated in splitless mode. The GC oven temperature was initially set at 50 °C for 5 minutes, followed by a gradual increase to 220 °C at a rate of 3 °C/min, where it was held for 5 minutes. Electron ionization (EI) at 70 eV was used, and mass spectra were recorded across a scan range of 30–500 m/z (3.1 scans per second).

Compound identification was performed using the NIST 14 mass spectral library (National Institute of Standards and Technology, Gaithersburg, MD, USA), with a match factor threshold of ≥85%. Retention indices (RI) were calculated and cross-referenced with literature values. Mass Hunter Qualitative Analysis Workflows (Agilent) software facilitated data processing. The relative quantities of volatile compounds were determined based on the proportion of individual peak areas to the total peak area. Identifications were further validated through comparison with n-alkane standards, library spectra, and existing retention index data.

2.9. Data analysis

All experiments, including total phenolic content, total flavonoid content, FRAP, and CUPRAC analyses, were conducted in triplicate. Samples for each plant organ were collected from three different individuals and pooled to form a single composite sample. These composite extracts were analyzed in triplicate, and the results are presented as means ± standard deviation. The data were statistically evaluated using one-way ANOVA in SPSS (version 27 for IBM SPSS Statistics), and group differences were assessed through Duncan's multiple range test, considering $p < 0.05$ as the threshold for significance.

3. Results and Discussion

3.1. Total Phenolic Content

The total phenolic content (TPC) of *C. macrocephala* extracts was assessed in this study, revealing significant variations among the plant's different parts. The highest TPC was observed in the leaves, with 7.88±0.32 mg GAE/g dry sample, followed by the stems at 2.05±0.85 mg GAE/g dry sample, and the flowers at 1.77±0.15 mg GAE/g dry sample (Table 1, Figure 2). These findings suggest that the leaves of *C. macrocephala* are particularly rich in phenolic compounds, which may be attributed to their critical roles in photosynthesis and defense against environmental stressors

(Shoeb et al., 2007; Khammar and Djeddi, 2012). A review of the existing literature highlighted the absence of previous studies reporting the total phenolic content of *C. macrocephala*, making this study the first to comprehensively analyze the phenolic profile of its leaves, flowers, and stems. This novel contribution is significant as it provides foundational data for understanding the chemical properties of *C. macrocephala* and its potential applications in phytochemical and pharmacological research. Although data on *Centaurea helenoides* and *Centaurea longifimbriata*, the closest relatives of *C. macrocephala* (Wagenitz, 1975), could not be identified in the literature, studies on other *Centaurea* species offer valuable comparisons. For instance, the phenolic content in the flowers of *Centaurea cyanus* has been reported to range between 1.5 and 2.0 mg GAE/g, which aligns closely with the values observed in *C. macrocephala* flowers (Khammar and Djeddi, 2012). Additionally, research on *C. calcitrapa* demonstrated that the leaves contain higher TPC levels compared to the stems and flowers, a pattern consistent with the results of this study (Shoeb et al., 2007). These similarities may indicate a chemotaxonomic trend across the genus. Leaves are known to accumulate more phenolic compounds due to their exposure to sunlight and the associated oxidative stress.

The differences in phenolic content among the various plant parts are likely influenced by their distinct physiological roles. Leaves, as the primary site of photosynthesis, tend to accumulate higher levels of phenolic compounds, which serve as antioxidants to protect against UV radiation and herbivory (Del Valle et al., 2020; Kumar et al., 2020; Emus-Medina et al., 2023). Flowers, in contrast, prioritize reproduction over defense, resulting in comparatively lower phenolic content (Stanković et al., 2014; Tyagi and Tyagi, 2017). Environmental factors such as altitude, soil quality, and climatic conditions also play a crucial role in shaping the phenolic profiles of plants, potentially accounting for the observed variations within *C. macrocephala* and across other *Centaurea* species (Kabtni et al., 2020; Salomon et al., 2021; Gülsoy et al., 2023).

Table 1. Total phenolic content, total flavonoid content, and antioxidant activities (FRAP and CUPRAC assays) of *C. macrocephala* extracts.

Taxon	Used part	Total phenolic content (mg GAE/g dry sample)*	Total flavonoid content (mg QE/g dry sample)*	FRAP (µmol $FeSO_4.7H_2O$/g sample)*	CUPRAC (mmol TEAC/g sample)*
C. macrocephala	Leaf	7.88±0.32^a	3.07±0.13^a	8.48±0.13^a	0.09±0.00^a
	Flower	1.77±0.15^b	0.76±0.05^b	6.50±0.06^b	0.02±0.00^b
	Stem	2.05±0.85^c	0.51±0.03^c	5.45±0.29^c	0.02±0.00^b

* The results are presented as mean ± standard deviation (SD). Different letters indicate significant differences according to Duncan's test at $p<0.05$.

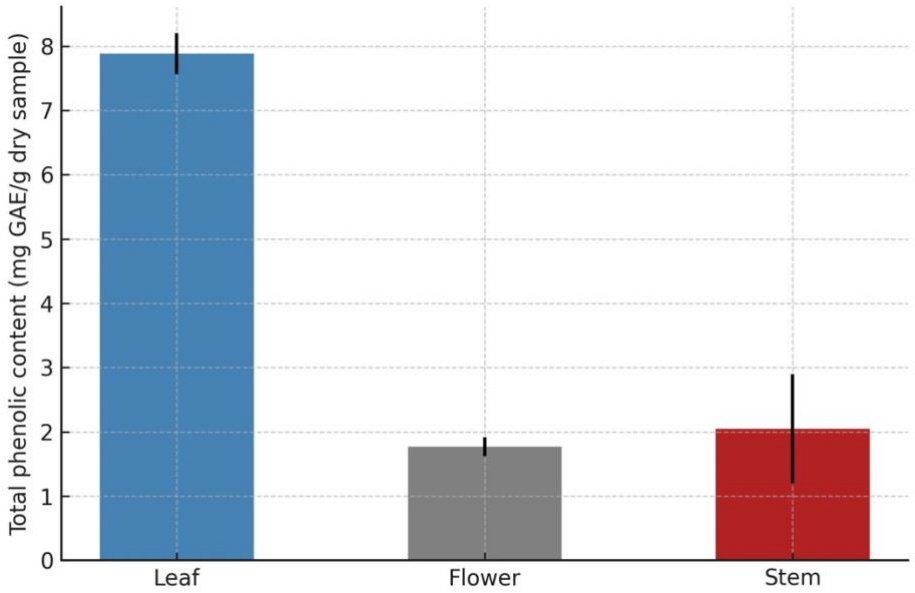

Figure 2. Total phenolic content (mg GAE/g dry sample) in the leaf, flower, and stem extracts of *C. macrocephala*. Error bars represent standard deviation (n = 3).

3.2. Total Flavonoid Content

The total flavonoid content (TFC) of *C. macrocephala* extracts was systematically evaluated, and statistically significant differences were identified among different parts of the plant. The highest TFC was measured in the leaves, with 3.07±0.13 mg QE/g dry sample. This was followed by the flowers (0.76±0.05 mg QE/g) and the stems (0.51±0.03 mg QE/g) (Table 1, Figure 3). These differences were found to be statistically significant ($p<0.05$). This distribution pattern may reflect a balance between biosynthetic capacity and the defensive needs of photosynthetically active plant organs (Shad et al., 2020; Shomali et al., 2022; Semenova et al., 2024).

Comparative analyses with existing literature reveal that data on the flavonoid profile of *Centaurea macrocephala* are considerably limited. Moreover, the lack of comprehensive studies on closely related species such as *C. helenoides* and *C. longifimbriata* further highlights the scarcity of information within this taxonomic group. Given the limited flavonoid data available for this species, these findings offer novel insights that contribute to the chemotaxonomic understanding of the genus *Centaurea*.

When other *Centaurea* species are considered, most analyses have been conducted on whole aerial parts, without distinction at the plant part level. Reported TFC values for *C. calcitrapa* and *C. cyanus* indicate moderate flavonoid concentrations, with leaves exhibiting higher flavonoid content than flowers. This may result from the reproductive focus of flowers and the defensive function of leaves (Stanković et al., 2014; Tyagi and Tyagi, 2017). This trend is consistent with the findings obtained for *C. macrocephala*.

The variations in TFC observed among different plant parts are thought to result from physiological roles of the organs, as well as environmental and biochemical factors. As the main site of photosynthesis, leaves tend to accumulate high levels of flavonoids to protect against UV radiation, neutralize free radicals, and defend against pathogens (Del Valle et al., 2020; Kumar et al., 2020; Emus-Medina et al., 2023). Higher expression of key enzymes involved in flavonoid biosynthesis in the leaves forms the biochemical basis of this accumulation (Treutter, 2005). In contrast, flowers, which are more focused on reproduction than on secondary metabolite production, generally contain lower flavonoid levels (Giorgi et al., 2010). Additionally, environmental variables such as altitude and soil composition may also influence flavonoid biosynthesis, contributing to the observed differences (Dolkar et al., 2017).

In our HPLC analyses of this study, rutin and quercetin derivatives were found to be predominant in the leaves, and this may be considered a major factor contributing to the total flavonoid content and antioxidant capacity. In conclusion, this study fills a significant gap in the literature regarding the flavonoid content of *C. macrocephala* and enables a comparative assessment of flavonoid levels in different plant parts. The data obtained provide fundamental information on the species' capacity for phenolic compound accumulation and organ-specific chemical differences, offering a basis for future phytochemical and pharmacological studies.

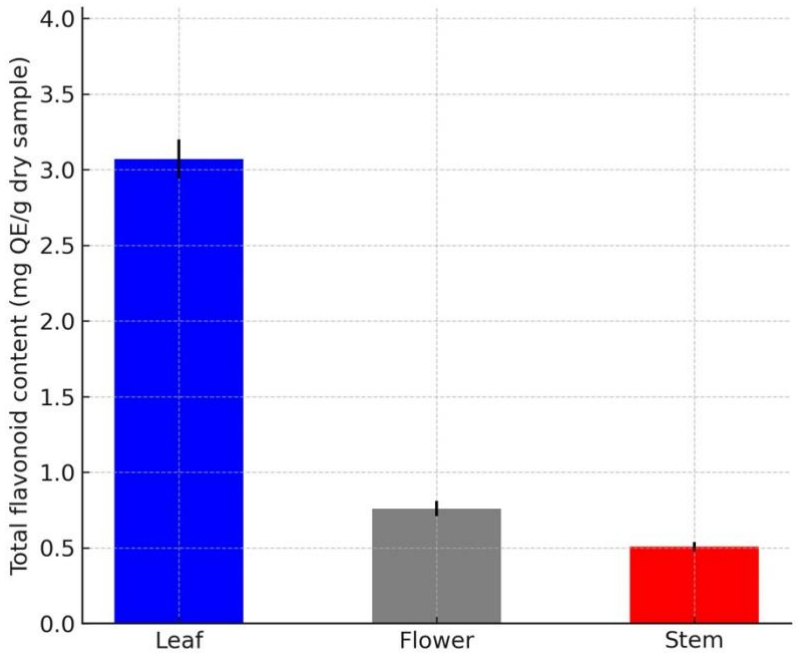

Figure 3. Total flavonoid content (mg QE/g dry sample) in different organs (leaf, flower, and stem) of *C. macrocephala*. Error bars represent standard deviation (n = 3).

3.3. Ferric Reducing Antioxidant Power (FRAP)

The Ferric Reducing Antioxidant Power (FRAP) values of *C. macrocephala* extracts showed statistically significant differences among the plant's different anatomical parts ($p<0.05$). The highest FRAP activity was measured in the leaves at 8.48±0.13 µmol $FeSO_4.7H_2O$/g dry sample, followed by the flowers (6.50±0.06 µmol $FeSO_4.7H_2O$/g) and the stems (5.45±0.29 µmol $FeSO_4.7H_2O$/g) (Table 1, Figure 4). These results indicate that the leaves of *C. macrocephala* are more active in terms of antioxidant capacity compared to other parts of the plant.

Similar trends have also been reported in species such as *C. cyanus* and *C. calcitrapa*. In studies conducted on these species, the leaves exhibited higher FRAP activity than both the flowers and stems (Stanković et al., 2014; Tyagi and Tyagi, 2017). These findings align with previously reported trends in the genus, reinforcing the organ-specific nature of antioxidant distribution.

It is known that antioxidant activity in plant organs is associated with organ-specific physiological functions and responses to environmental conditions. Since leaves are exposed to high levels of reactive oxygen species during photosynthesis, they may synthesize greater amounts of antioxidant compounds (e.g., phenolics, flavonoids) for free radical neutralization. (Giorgi et al., 2010; Kabtni et al., 2020). Additionally, it has been reported that the expression of key enzymes involved in flavonoid biosynthesis is higher in leaves (Treutter, 2005). In contrast, floral and stem organs, being more focused on reproductive and structural functions, may have a more limited antioxidant defense capacity.

In conclusion, this study presents the first comprehensive and statistically supported comparative evaluation of FRAP activity in the leaves, flowers, and stems of *C. macrocephala*. The data obtained reveal that the antioxidant capacity of the species differs significantly between organs, with leaves standing out in terms of phenolic compound accumulation. These findings provide fundamental information on the organ-level antioxidant profile of *C. macrocephala* and may serve as a reference point for future phytochemical and pharmacological research. Furthermore, the results may serve as preliminary information for studies aiming to evaluate plant-based antioxidant sources. Further investigations may enhance our understanding of the biological relevance of these findings.

3.4. Copper Ion Reduction Assay (CUPRAC)

The Copper Reducing Antioxidant Capacity (CUPRAC) values of *C. macrocephala* extracts exhibited statistically significant differences among different plant parts ($p<0.05$). The leaves showed the highest reducing activity, with a value of 0.09±0.00 mmol TEAC/g dry sample. In contrast, both

the flowers and stems displayed lower CUPRAC activities, each measured at 0.02±0.00 mmol TEAC/g dry sample (Table 1, Figure 4).

Compared to other antioxidant assays, CUPRAC results revealed a more pronounced difference between the leaves and other anatomical structures, underscoring its sensitivity to subtle variations in reducing capacity. The higher CUPRAC values in the leaves may reflect the presence of flavonoid classes known to form stable complexes with Cu^{2+} ions, thereby enhancing their reducing capacity. Notably, compounds such as quercetin, rutin, catechin, and chlorogenic acid—commonly identified in *Centaurea* species—have been reported to exhibit strong copper ion reducing capacity in CUPRAC assays (Apak et al., 2008; Özyürek et al., 2011).

The variation in CUPRAC activity may not only reflect the quantitative presence of antioxidant compounds but could also be associated with the functional roles and stress responsiveness of individual plant organs. Leaves, as physiologically more active organs, are more engaged in sensing environmental cues and producing defensive metabolites. This characteristic may contribute to the accumulation of a broader range of antioxidant compounds in leaves, thereby enhancing their CUPRAC activity. In contrast, floral and stem organs, which primarily serve reproductive or structural functions, may possess a comparatively lower capacity for antioxidant accumulation.

Previous studies on *Centaurea* species align with our observations. For instance, Aktumsek et al. (2013) reported that the methanolic extract of *Centaurea pulcherrima* exhibited higher CUPRAC activity in aerial parts compared to flowers and stems. Similarly, Zengin & Aktumsek (2010) found that leaf extracts of *Centaurea urvillei* had superior cupric ion reducing capacity. These findings reinforce our data showing enhanced CUPRAC activity in *C. macrocephala* leaves. The specific redox mechanism of the CUPRAC assay may further contribute to the marked variation observed between plant organs, particularly favoring copper-reactive compounds (Giorgi et al., 2010; Kabtni et al., 2020). The distinct redox chemistry of the CUPRAC method—based on the reduction of Cu^{2+} ions—may also contribute to the enhanced sensitivity observed in leaves. Unlike FRAP, which relies on Fe^{3+} reduction and is more selective for hydrophilic antioxidants, CUPRAC can interact with a broader spectrum of antioxidant classes, including those forming stable Cu^{2+} complexes. This broader chemical compatibility may help amplify organ-specific differences, especially in favor of flavonoid-enriched leaves. Therefore, the observed divergence between FRAP and CUPRAC responses might reflect not only compound abundance but also assay-specific selectivity and redox interaction dynamics.

This study presents the first comprehensive evaluation of CUPRAC activity in *C. macrocephala* extracts. The findings contribute to the current understanding of organ-level antioxidant variability within the genus *Centaurea* and establish a foundation for future phytochemical research focusing on phenolic compound distribution. Additionally, these data may serve as a preliminary reference for

studies aimed at developing plant-based antioxidant formulations that consider the differential antioxidant potential of distinct plant parts.

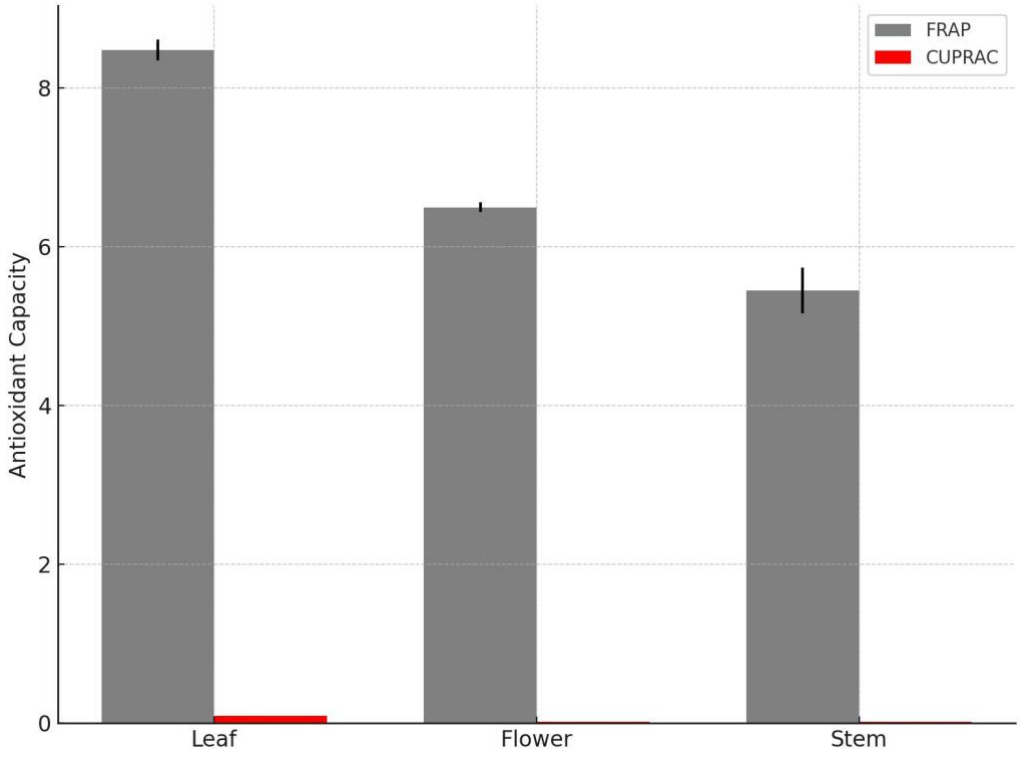

Figure 4. Comparative evaluation of FRAP and CUPRAC antioxidant capacities in different organs (leaf, flower, and stem) of *C. macrocephala*. Results are expressed as mean ± SD (n = 3).

3.5. Analysis of Bioactive Compounds via HPLC

The HPLC analysis of *C. macrocephala* revealed a rich phenolic composition with considerable variations in flavonoids, phenolic acids, and lignans across the extracts obtained from different plant parts. Among the most abundant phenolic compounds were chlorogenic acid, rutin, quercetin derivatives, and caffeic acid, whose concentrations varied between the flower, leaf, and stem extracts (Table 2). In particular, compounds such as 3,4-dihydroxybenzoic acid (114.81 mg/L), chlorogenic acid (153.1 mg/L), and myricetin (83.29 mg/L) were detected at notably high levels in the leaves (Table 2). These compounds are reported in the literature to possess antioxidant, anti-inflammatory, and cytoprotective potential (Grace and Logan, 2000; Harborne, 1993; Badaoui et al., 2024). The present findings provide new insights into their organ-specific distribution in this species.

Chlorogenic acid is known for its free radical scavenging ability and plays an active role in plant defense responses. Similarly, myricetin has been shown to exhibit antioxidant and anti-inflammatory effects, and has also been associated with anticancer and cardioprotective properties in some studies (Badaoui et al., 2024). 3,4-Dihydroxybenzoic acid is recognized for its radical-

scavenging capabilities and potential antimicrobial activity. The accumulation of these compounds specifically in the leaves suggests that this part may be particularly important in terms of biological activity.

The results are also in line with prior phytochemical investigations on the genus *Centaurea*. Litvinenko & Bubenchikova (1988) reported the presence of flavonoid aglycones—luteolin, apigenin, quercetin, kaempferol, and isorhamnetin—in *C. cyanus*, alongside caffeic, chlorogenic, neochlorogenic, and isochlorogenic acids. These flavonoids are well-known for their antioxidant and cytoprotective effects, and their localization in leaves and flowers likely reflects adaptive functions related to oxidative stress defense and ecological interactions. The confirmed presence of caffeic acid and its ester 3-O-feruloylquinic acid further underscores the significant role of hydroxycinnamic acid derivatives in the secondary metabolism of *C. macrocephala* (Shoeb et al., 2007). Such phenolic acids act as intermediates in the phenylpropanoid pathway, contributing to lignin biosynthesis, cell wall fortification, photoprotection, and defense against pathogens (Clifford, 1999; Boerjan et al., 2003; Grace & Logan, 2000; Harborne, 1993).

The detection of caffeic acid derivatives in *C. macrocephala*, which are also known for their allelopathic properties, suggests that this species may employ chemical defense mechanisms in its ecological interactions (Hierro and Callaway, 2021). Furthermore, lignans such as matairesinoside, arctiin, and matairesinol, previously identified in the seeds of this species, indicate a potential link between the phenylpropanoid pathway and lignan biosynthesis, and support the hypothesis that these compounds may be associated with antioxidant and cytotoxic effects (Shoeb et al., 2005; Shoeb et al., 2007).

The differences in the phenolic profile of *C. macrocephala* compared to other species demonstrate species-level variation in secondary metabolites, which may be attributed to ecological or genetic factors. For example, sesquiterpene lactones such as cnicin have been reported as dominant in *C. calolepis* (Baykan Erel et al., 2011), whereas no such compounds were detected in the present study. This suggests that distinct metabolic pathways may be active in the phenolic biosynthesis of different species. The current findings indicate that the notable intra- and interspecific differences in phenolic profiles may serve as potential chemotaxonomic markers within the genus *Centaurea*. However, confirming the chemotaxonomic relevance of phenolic compounds will require broader and more systematic comparisons across multiple species. Nevertheless, this study offers a valuable reference dataset, particularly given the scarcity of data on *C. macrocephala* in the current literature.

Ascorbic acid (vitamin C) was included among the target compounds in the HPLC analysis performed in this study. However, it was not detected in the flower, leaf, or stem extracts under the applied analytical conditions, and therefore was marked as “N/D” (Not Detected) in the related table. In the literature, it has been widely reported that ascorbic acid is highly sensitive to light, heat, and

oxidative conditions, which may cause rapid degradation during extraction and analytical procedures (Arrigoni & De Tullio, 2000; Eitenmiller et al., 2008). This may explain why ascorbic acid could not be detected, possibly due to its instability during sample preparation or HPLC injection.

Table 2. Quantification of Phenolic Compounds in Different Organs (Leaf, Flower, and Stem) of *C. macrocephala*

No	Compounds	*C. macrocephala*		
		Flower (mg/L)	Leaf (mg/L)	Stem (mg/L)
	Vitamin C			
1	Ascorbic acid	N/D	N/D	N/D
	Phenolics			
2	Gallic acid	21.37	40.40	35.81
3	3,4-Dihydroxybenzoic acid	29.49	114.81	105.2
4	Vanillic acid	N/D	840.44	98.26
5	Syringic acid	N/D	N/D	N/D
6	Coumaric acid	N/D	N/D	N/D
7	Caffeic acid	N/D	3.16	N/D
8	Ferulic acid	N/D	N/D	N/D
9	Rosmarinic acid	11.32	14.88	5.88
10	Progallol	N/D	110.57	N/D
11	Chlorogenic acid	61.09	153.1	58.02
12	Resveratrol	N/D	N/D	N/D
13	Oleuropein	N/D	N/D	N/D
	Flavonoids			
14	Catechin	N/D	N/D	N/D
15	Epicatechin	93.06	N/D	49.18
16	Rutin	2.72	297.42	15.47
17	Myricetin	25.66	83.29	N/D
18	Quercetin	52.0	21.95	22.99
19	Apigenin	N/D	N/D	11.02
20	Cyanidin chloride	N/D	N/D	N/D
22	Hesperitin	1.12	N/D	N/D
23	Kaempferol	N/D	N/D	N/D
24	Baicalin	10.36	0.2	N/D
25	Chrysin	N/D	N/D	N/D

N/D: Not Detected

Furthermore, some studies on *Centaurea* species within the Asteraceae family have reported that ascorbic acid can be detected at low levels. For instance, Petropoulos et al. (2020) analyzed *Centaurea raphanina* subsp. *mixta* and reported relatively low concentrations of ascorbic acid.

Similarly, Mekky et al. (2024) detected limited amounts of ascorbic acid in *Centaurea calcitrapa*. These findings suggest that ascorbic acid may occur in *Centaurea* species at limited levels and that the "N/D" result obtained in the present study is consistent with the literature in terms of both biological distribution and analytical sensitivity.

In conclusion, this study provides a detailed overview of the phenolic distribution in the leaf, flower, and stem of *C. macrocephala*, offering essential insights into the organ-specific phytochemical composition of the species. The high concentrations of 3,4-dihydroxybenzoic acid, chlorogenic acid, and myricetin in the leaf extracts suggest that this part may stand out in terms of antioxidant potential. The known biological activities of these compounds may form a basis for future investigations evaluating the therapeutic relevance of *C. macrocephala*. Moreover, the present data contribute to the understanding of interspecific phenolic variation and enhance the existing literature on the chemical diversity within the genus *Centaurea*.

3.6. GC-MS-SPME Analysis of Volatile Compounds

This section presents the first assessment of the volatile compound profile of *C. macrocephala* using the GC-MS-SPME technique. This method enables the analysis of organ-specific volatile emissions without solvent use, providing a more ecologically relevant representation of plant responses to environmental conditions.

The volatile analysis showed that the profile of *C. macrocephala* is dominated by aldehydes and ketones, a pattern that differs from certain other *Centaurea* species. For instance, terpenoid compounds such as hexadecanoic acid and carvacrol have been reported as dominant in *C. calolepis* (Baykan Erel et al., 2011), while *Centaurea dissecta* is known to be rich in sesquiterpene lactones (Mishio et al., 2014). These differences suggest species-level variation in volatile compound orientation within the genus.

Hexanal was the most abundant compound across all plant parts, detected at 86.28% in the flowers, 66.61% in the stem, and 51.25% in the leaves (Table 3, Figure 5). Benzaldehyde was also present in noteworthy amounts, with 2.97% in the stem and 6.85% in the leaves. Additionally, 2-n-pentylfuran was found exclusively in the flower (7.31%), suggesting a flower-specific function for this compound. However, its ecological role remains insufficiently studied. The detection of 1-octen-3-ol in the leaves and matsutake alcohol in the stem further supports the idea that different plant parts produce distinct volatile components (Table 3). In addition to individual compound levels, the overall distribution of volatile constituents across different organs is visualized in Figure 6, which highlights

the proportional richness of volatile metabolites in the flower, stem, and leaf. This representation underscores the floral tissue as the most dominant in terms of total volatile content, particularly due to its exceptionally high hexanal concentration.

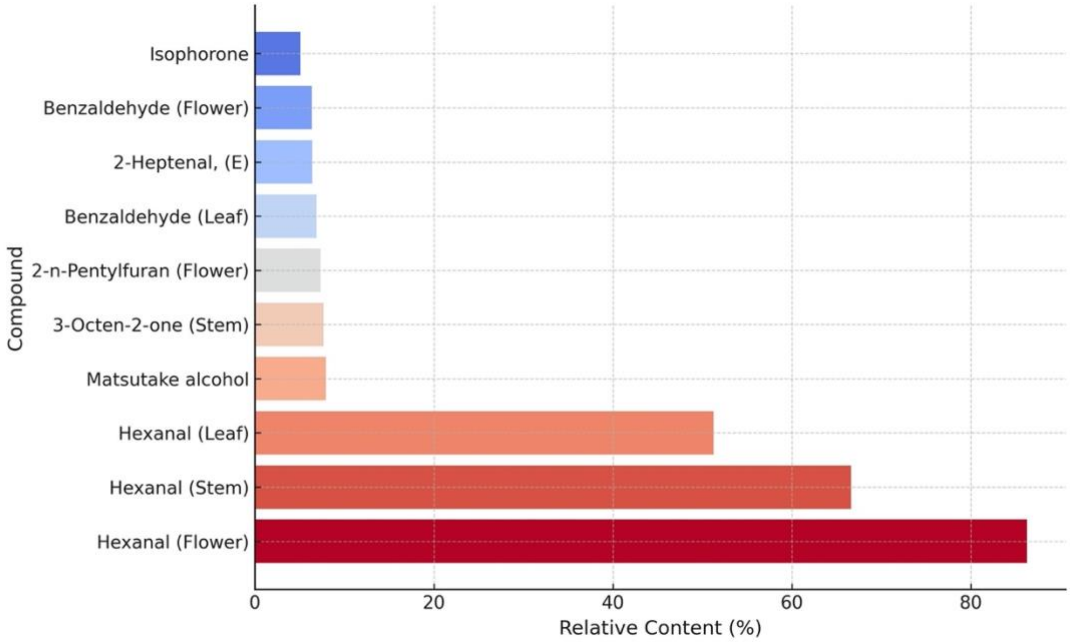

Figure 5. Relative content (%) of the most abundant volatile compounds identified in different organs (flower, leaf, and stem) of *C. macrocephala* based on GC-MS analysis.

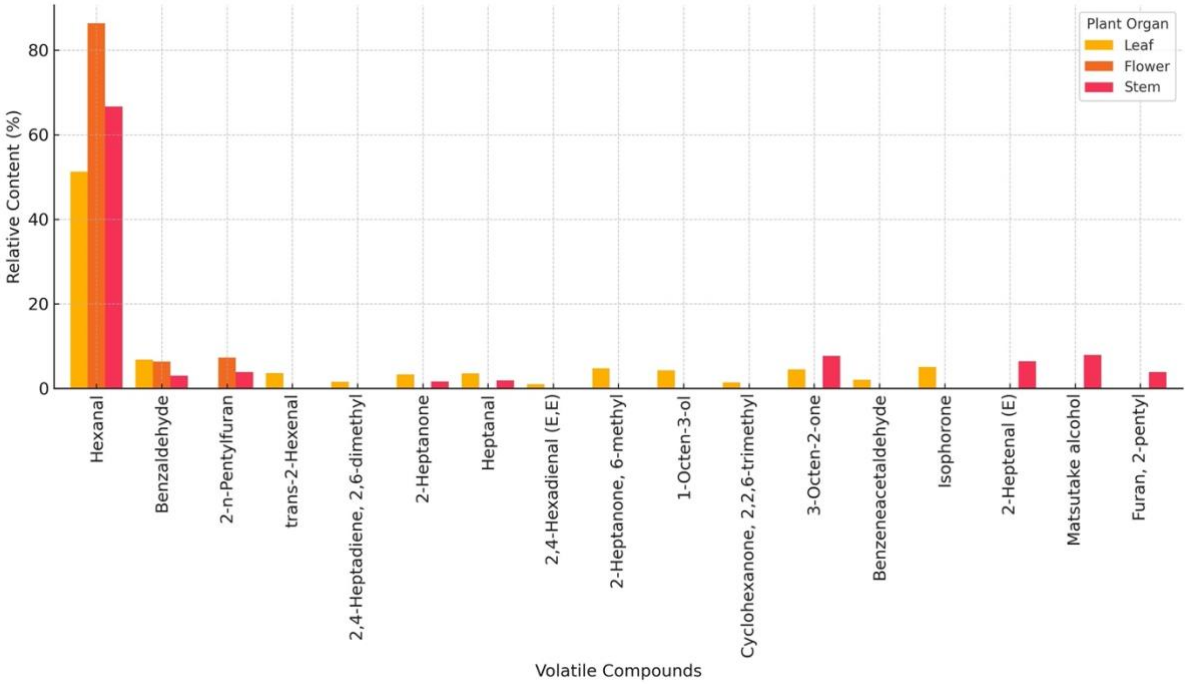

Figure 6. Total volatile compound distribution (%) identified by GC-MS analysis in different plant organs (flower, leaf, and stem) of *C. macrocephala*.

Among the ketone group, 3-octen-2-one was identified at 4.53% in the leaves and 7.67% in the stem. 2-heptanone was found in lower proportions in both parts (3.31% and 1.68%, respectively). Most of the detected aldehydes and ketones are believed to originate from lipid oxidation. These compounds are typically derived from the oxidative breakdown of fatty acids via the lipoxygenase (LOX) pathway.

The data indicate that the volatile profile of *C. macrocephala* diverges significantly from certain other *Centaurea* species. For example, in a study on *Centaurea pamphylica*, a profile rich in lignans and sesquiterpene lactones was reported, although low levels of ketones such as 2-heptanone and oxygenated aldehydes were also detected (Shoeb et al., 2007). The presence of 2-heptanone in both species suggests it may be a widespread, though quantitatively variable, component within the genus. While *C. pamphylica* exhibits a sesquiterpene-dominant profile, *C. macrocephala* is characterized by its aldehyde and ketone-rich composition, pointing to distinct pathways in secondary metabolism.

Similarly, terpenoid compounds such as hexadecanoic acid and carvacrol are predominant in *C. calolepis* (Baykan Erel et al., 2011), whereas these compounds were not detected in *C. macrocephala*. *C. dissecta* is rich in sesquiterpene lactones and flavonoids (Mishio et al., 2014). These findings support the idea of species-specific orientations in secondary metabolism within the genus *Centaurea*.

The potential ecological functions of the identified volatile compounds are also noteworthy. Hexanal, recognized as a green leaf volatile (GLV), plays a role in plant defense by deterring herbivores and pathogens (Shoeb et al., 2005). Benzaldehyde, an aromatic compound, contributes to floral scent and has been associated with pollinator attraction in various plant species (Badaoui et al., 2024). The presence of 2-n-pentylfuran exclusively in the flower may suggest a role in stress response or herbivore deterrence. However, determining its specific function requires further biological studies.

In conclusion, this study provides a detailed characterization of the volatile diversity in the leaf, flower, and stem of *C. macrocephala*, offering meaningful insights into its secondary metabolite profile. The predominance of aldehydes and ketones suggests that *C. macrocephala* differs chemically from some other *Centaurea* species and may contribute to chemotaxonomic evaluations. Additionally, the findings support future studies investigating organ-specific biosynthesis of volatiles and their potential roles in ecological interactions.

Overall, the data indicate that *C. macrocephala* is capable of producing biologically relevant volatile constituents, offering preliminary information for its potential application in areas such as natural fragrance sources or volatile antioxidant raw materials. However, compound-level bioactivity and bioavailability tests are required to validate this potential.

Table 3. Identified volatile constituents of *C. macrocephala* (%) in flower, leaf and stem by GC-MS analysis.

Plant part	Constituents	RT (min)	RI	Content [%]
Flower	Hexanal	6.148	801	86.280
	Benzaldehyde	12.401	960	6.342
	2-n-Pentylfuran	13.97	993	7.312
Leaf	Hexanal	6.137	801	51.254
	trans-2-Hexenal	7.850	849	3.612
	2,4-Heptadiene, 2,6-dimethyl	8.1	856	1.558
	2-Heptanone	9.3	890	3.314
	Heptanal	9.72	902	3.573
	2,4-Hexadienal, (E,E)	10.1	910	1.032
	2-Heptanone, 6-methyl	12.165	953	4.754
	Benzaldehyde	12.374	958	6.851
	1-Octen-3-ol	13.315	977	4.253
	Cyclohexanone, 2,2,6-trimethyl	16.15	1038	1.423
	3-Octen-2-one	16.407	1044	4.530
	Benzeneacetaldehyde	16.615	1048	2.027
	Isophorone	17.477	1072	5.070
Stem	Hexanal	9.304	890	66.61
	2-Heptanone	9.726	902	1.685
	Heptanal	12.192	954	1.899
	2-Heptenal, (E)	12.385	958	6.4
	Benzaldehyde	13.315	977	2.978
	Matsutake alcohol	13.941	991	7.930
	Furan, 2-pentyl	16.402	1084	3.888
	3-Octen-2-one	9.304	890	7.677

Rt - Retention times on an HP-5MS UI column

RI - Experimentally determined retention indices on an HP-5MS UI column

3.7. Chemotaxonomic implications

This study presents chemotaxonomic evaluations based on the phenolic and volatile compound profiles of *C. macrocephala*, aimed at assessing its taxonomic position within the genus. The genus *Centaurea* is known for its diverse array of secondary metabolites, including phenolic acids, flavonoids, lignans, sesquiterpene lactones, and volatile components (Sarker et al., 1997; Shoeb et al., 2005). The structural diversity of these metabolites across species makes chemical profiling a valuable tool in intra-generic classification. In particular, flavonoids and their glycosides are considered reliable chemotaxonomic markers due to their phylogenetic distinctiveness and ecological functions (De Oliveira et al., 2017).

HPLC analysis of *C. macrocephala* highlighted the predominance of hydroxycinnamic acid derivatives, flavonols, and lignans. Chlorogenic acid, rutin, quercetin derivatives, and caffeic acid were the most abundant compounds. The relatively high level of chlorogenic acid compared to other

phenolic acids is notable in terms of the species' inclination toward phenylpropanoid metabolism. The dominance of rutin and quercetin glycosides also supports the role of flavonols in chemical defense. These findings align with previous reports describing luteolin, apigenin, and kaempferol derivatives as common flavonoids in *Centaurea* species (Mishio et al., 2015).

The identification of 3-O-feruloylquinic acid in this study, also previously reported in *C. cyanus* and *C. calcitrapa*, indicates that hydroxycinnamic acid derivatives are widespread and potentially significant for taxonomic differentiation within the genus (Badaoui et al., 2024). The distinct phenolic profile of *C. macrocephala*, as compared to sesquiterpene-rich species such as *C. calolepis*, may reflect divergent biosynthetic orientations among species (Baykan Erel et al., 2011).

Comparative data suggest that *C. macrocephala* synthesizes quercetin glycosides and hydroxycinnamic acid derivatives preferentially, whereas species such as *C. cyanus* and *C. calcitrapa* are richer in anthocyanins and flavanones. These chemical differences may indicate species-specific adaptations related to pigmentation and stress responses (Curkovic-Perica et al., 2014).

The detection of apigenin, quercetin, and luteolin derivatives in *C. macrocephala* is consistent with findings in *Centaurea arenaria*, *C. cyanus*, *Centaurea jacea*, *Centaurea montana*, and *Centaurea scabiosa* (Negrete et al., 1988; Christensen, 1991). However, methylated flavones such as apigenin 7,4'-dimethyl ether, reported in some *Centaurea* species, were not identified in the present study. This may suggest a metabolic preference for phenolic acid production over flavonoid methylation in *C. macrocephala*, although broader comparative data are required to validate this observation (De Oliveira et al., 2017).

The presence of lignans such as matairesinoside and syringaresinol suggests a potential biosynthetic link between phenylpropanoid metabolism and lignan biosynthesis. Structural diversity in lignans may offer chemotaxonomic insights at the species level (Csapi et al., 2010).

GC-MS-SPME analysis revealed that the volatile profile of *C. macrocephala* is dominated by aldehydes and ketones, differing from sesquiterpene-rich species such as *C. pamphylica* and *C. calolepis* (Shoeb et al., 2007). The presence of hexanal and benzaldehyde aligns its profile more closely with aldehyde-rich species such as *C. cyanus* and *C. calcitrapa* (Baykan Erel et al., 2011).

2-n-Pentylfuran was detected exclusively in the floral organ and has been associated with oxidative stress responses in other plant systems (Badaoui et al., 2024). The identification of ketones such as 3-octen-2-one and 2-heptanone, also reported in other *Centaurea* species with limited sesquiterpene production, suggests that these compounds may contribute to species-specific volatile profiles (Shoeb et al., 2007; Baykan Erel et al., 2011).

From a chemotaxonomic perspective, the low levels of monoterpenes and sesquiterpenes in *C. macrocephala* suggest a metabolic trajectory favoring lipid-derived volatiles and flavonoid-based defense compounds. For example, germacrene D and caryophyllene oxide are dominant in *C. dissecta*

and *Centaurea virgata* (Kilic, 2013), whereas they were not detected in this species. These differences may be influenced by genetic or environmental factors affecting secondary metabolism.

In summary, the phytochemical characteristics of *C. macrocephala*, including its flavonoid- and aldehyde-rich profile, distinguish it from sesquiterpene-dominant taxa. These traits point to a chemotaxonomic orientation that favors phenylpropanoid-derived metabolic pathways.

To more clearly and reliably resolve the taxonomic status of *C. macrocephala*, large-scale phylogenetic analyses incorporating molecular data and metabolomic profiling involving multiple closely related taxa are warranted.

4. Conclusions and Recommendations

This study conducted a comparative analysis of the phenolic, flavonoid, antioxidant, and volatile compound profiles in different parts (flower, leaf and stem) of *C. macrocephala*, providing significant insights into the species' phytochemical diversity.

The results indicate that leaves exhibit the highest levels of phenolic and flavonoid content, which is associated with strong antioxidant activity, particularly in FRAP and CUPRAC assays. HPLC analysis identified chlorogenic acid, rutin, and quercetin derivatives as the predominant phenolic compounds. Volatile compound analysis (GC-MS-SPME) revealed that the volatile profile is mainly composed of aldehydes and ketones, distinguishing *C. macrocephala* from sesquiterpene-rich *Centaurea* species.

The structural diversity of phenolic compounds suggests that *C. macrocephala* may exhibit a metabolic tendency toward phenylpropanoid-derived pathways. The use of solvent-free SPME-GC-MS provided refined data on the species' volatile profile, contributing to a better understanding of its phytochemical structure and allowing for the consideration of possible ecological associations in future research.

The high levels of chlorogenic acid, rutin, and quercetin derivatives, along with certain aldehydes and ketones detected in this study, have been associated with antioxidant and cytoprotective properties in previous literature. These findings suggest that *C. macrocephala* may be considered a potential candidate for plant-based applications, particularly in antioxidant-related fields. Nevertheless, to substantiate these preliminary findings and better understand their functional relevance, future studies should focus on the bioavailability and biological activity of the identified compounds through advanced in vivo and in vitro assays.

This study significantly enriches the limited phytochemical data available for *C. macrocephala* and contributes to the understanding of its biological potential. The findings, particularly the richness

in antioxidant compounds and distinct chemotaxonomic characteristics, provide a valuable foundation for future pharmacological, molecular, and taxonomic research.

To more reliably clarify the taxonomic position of *C. macrocephala* and comprehensively evaluate its biological activity, integrated research approaches supported by molecular phylogenetic data and metabolomic analysis are needed.

This study provides pioneering insights into the antioxidant capacity and phytochemical profile of *C. macrocephala*. The data obtained establish a foundational reference for the species' biological potential, and future studies incorporating advanced biological assays—such as antimicrobial, anti-inflammatory, or cytotoxic evaluations—will not only enable a more holistic assessment of its therapeutic effects but also help to better elucidate its pharmacological and ethnobotanical significance.

Acknowledgements

The author sincerely thank Dr. Abidin GUMRUKCUOGLU for sharing their expertise in HPLC and GC-MS-SPME analyses, which greatly contributed to the successful execution of this study.

Authors' Contributions

The author was responsible for the conception and design of the study, data collection, analysis and interpretation of the data, and drafting the manuscript.

Statement of Conflicts of Interest

There is no known conflict of interest.

Statement of Research and Publication Ethics

The author declares that this study complies with Research and Publication Ethics.

References

Apak, R., Güçlü, K., Özyürek, M., and Çelik, S. E., (2008). Mechanism of antioxidant capacity assays and the CUPRAC (cupric ion reducing antioxidant capacity) assay. *Microchimica acta*, 160(4), 413-419.

Aktumsek, A., Zengin, G., Guler, G. O., Cakmak, Y. S., and Duran, A., (2013). Antioxidant potentials and anticholinesterase activities of methanolic and aqueous extracts of three endemic *Centaurea* species. *Food and Chemical Toxicology*, 55, 290-296.

Arrigoni, O., and De Tullio, M. C., (2000). The role of ascorbic acid in cell metabolism: between chemistry and biology. *Journal of Plant Physiology*, 157(5), 481-488.

Baykan Erel, S., Karaalp, C., Bedir, E., Kaehlig, H., Glasl, S., Khan, S., and Krenn, L., (2011). Secondary metabolites of *Centaurea calolepis* and evaluation of cnicin for anti-inflammatory, antioxidant, and cytotoxic activities. *Pharmaceutical Biology,* 49(8), 840-849.

Baytop, T., (1999). *Therapy with medicinal plants in Turkey (Past and Present).* Istanbul: Istanbul University Publications.

Badaoui, M. I., Kilic, O., and Bagci, E., (2024). Chemical constituents of *Centaurea dissecta* Ten. and sesquiterpenes chemotaxonomic significance. *Biochemical Systematics and Ecology,* 114*,* 104808.

Benzie, I. F., and Strain, J. J., (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical Biochemistry,* 239(1), 70-76.

Boerjan, W., Ralph, J., and Baucher, M., (2003). Lignin biosynthesis. *Annual Review of Plant Biology,* 54*,* 519–546.

Clifford, M. N., (1999). Chlorogenic acids and other cinnamates—nature, occurrence, and dietary burden. *Journal of the Science of Food and Agriculture,* 79(3), 362–372.

De Oliveira, A. P., Brighente, I. M., and Pizzolatti, M. G., (2017). Flavonoids as chemotaxonomic markers in Asteraceae. *Phytochemistry,* 140*,* 1–14.

Del Valle, J. C., Buide, M. L., Whittall, J. B., Valladares, F., and Narbona, E., (2020). UV radiation increases phenolic compound protection but decreases reproduction in *Silene littorea*. *PloS one,* 15(6), e0231611.

Ekim, T., Koyuncu, M., Vural, M., Duman, H., Aytaç, Z., and Adıgüzel, N., (2012). *Centaurea* L. In T. Ekim (Ed.), *Türkiye Bitkileri Listesi (Damarlı Bitkiler)* (pp. 127-140). İstanbul: Nezahat Gökyiğit Botanik Bahçesi Yayınları ve Flora Araştırmaları Derneği.

Emus-Medina, A., Contreras-Angulo, L. A., Ambriz-Perez, D. L., Vazquez-Olivo, G., and Heredia, J. B., (2023). UV light stress induces phenolic compounds in plants. In R. Lone, S. Khan, and A. Mohammed Al-Sadi (Eds.), *Plant phenolics in abiotic stress management* (pp. 369–389). Springer, Singapore.

Grace, S. C., and Logan, B. A., (2000). Energy dissipation and radical scavenging by the plant phenylpropanoid pathway. *Philosophical Transactions of the Royal Society B: Biological Sciences,* 355(1402), 1499–1510.

Giorgi, A., Madeo, M., Speranza, G., and Cocucci, M., (2010). Influence of environmental factors on the composition of phenolic antioxidants of *Achillea collina Becker ex Rchb. Natural Product Research,* 24(14), 1546-1559.

Harborne, J. B., (1993). *Introduction to Ecological Biochemistry* (4th ed.). Academic Press.

Hierro, J. L., and Callaway, R. M., (2021). The ecological importance of allelopathy. *Annual Review of Ecology, Evolution, and Systematics,* 52(1), 25-45.

Kabtni, S., Sdouga, D., Rebey, I. B., Save, M., Trifi-Farah, N., Fauconnier, M. L., and Marghali, S., (2020). Influence of climate variation on phenolic composition and antioxidant capacity of *Medicago minima* populations. *Scientific Reports*, 10*,* 8293.

Kumar, S., Abedin, M. M., Singh, A. K., and Das, S., (2020). Role of phenolic compounds in plant-defensive mechanisms. In R. Lone, R. Shuab, and A. Kamili (Eds.), *Plant phenolics in sustainable agriculture* (pp. 509–530). Springer, Singapore.

Łazarski, R., and Pliszko, A., (2022). First spontaneous occurrence of *Centaurea macrocephala* in Poland. *BioInvasions Records,* 11(2), 330-337.

Litvinenko, V. I., and Bubenchikova, V. A., (1988). Flavonoid aglycones (quercetin, kaempferol, isorhamnetin, apigenin, luteolin, hispidulin) and their glycosides, and caffeic, chlorogenic, neochlorogenic, and isochlorogenic acids have been isolated from *Centaurea cyanus* L. *Chemistry of Natural Compounds*, 24, 792-795.

Mekky, A. E., Saied, E., Abdelmouty, E. S., Haggag, M. I., Khedr, M., Khalel, A. F., Al-Habibi, M. M., Metwally, S. A., Askary, A., E., Mohammad, A. M., Alshehri, W. A., Sharahili, A. I., Khairy, N. M., Abdelaziz, A. E. M., and Mahmoud, N. N., (2024). Phytochemical Analysis of *Centaurea calcitrapa* L. Aerial Flowering Parts Serial Solvent Extracts and Its Antibacterial and Antioxidant Activities. *Life*, 14(7), 900.

Mishio, T., Sugimoto, N., and Tanaka, K., (2014). Anthocyanins and other flavonoids as flower pigments from eleven *Centaurea* species. *Journal of Natural Products*, 77(6), 1215-1223.

Özyürek, M., Güçlü, K., Tütem, E., Başkan, K. S., Erçağ, E., Çelik, S. E., and Apak, R., (2011). Comprehensive review of CUPRAC methodology. *Analytical Methods*, 3(11), 2439-2453.

Pawliszyn, J., (1997). Solid Phase Microextraction: Theory and Practice. Wiley-VCH.

Petropoulos, A., Fernandes, S., Dias, Â., Pereira, M. I., Calhelha, C., Chrysargyris, R. C., Tzortzakis, A., Ivanov, N., Sokovic, M., Barros, M. D., and Ferreira, I. C. F. R., (2020). Chemical composition and plant growth of *Centaurea raphanina* subsp. *mixta* plants cultivated under saline conditions. *Molecules,* 25(9), 2204.

Pichersky, E., and Gershenzon, J., (2002). The formation and function of plant volatiles: perfumes for pollinator attraction and defense. *Current Opinion in Plant Biology*, 5(3), 237-243.

Prior, R. L., Wu, X., and Schaich, K., (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry,* 53(10), 4290-4302.

Özel, Ç. A., and Maesaroh, S., (2023). An outlook of the cultivation, medicinal properties, and tissue culture techniques of *Centaurea* in Türkiye: A Comprehensive Review. *MAS Journal of Applied Sciences*, 8(2), 362-373.

Sarker, S. D., Kumarasamy, Y., Shoeb, M., Celik, S., Eucel, E., Middleton, M., and Nahar, L., (2005). Antibacterial and antioxidant activities of three Turkish species of the genus *Centaurea*. *Advances in Traditional Medicine,* 5(3), 246-250.

Sarker, S. D., Latif, Z., and Gray, A. I., (1997). Natural products isolation. *Methods in Biotechnology*, 4*,* 1-25.

Sezik, E., Yeşilada, E., Honda, G., Takaishi, Y., Takeda, Y., and Tanaka, T., (2001). Traditional medicine in Turkey X. Folk medicine in central Anatolia. *Journal of ethnopharmacology*, *75*(2-3), 95-115.

Shad, M. A., Shahid, M., and Saeed, H., (2020). Effects of environmental stress on antioxidant properties and phenolic contents in different plant parts. *Agronomy,* 10(1209).

Shoeb, M., (2005). *Cytotoxic compounds from the genus* Centaurea (Doctoral dissertation). Retrieved from https://openair.rgu.ac.uk/

Shoeb, M., MacManus, S. M., Kong-Thoo-Lin, P., Celik, S., Jaspars, M., Nahar, L., and Sarker, S. D., (2007). Bioactivity of the extracts and isolation of lignans and a sesquiterpene from the aerial parts of *Centaurea pamphylica* (Asteraceae). *DARU Journal of Pharmaceutical Sciences*, *15*(3), 118-122.

Shomali, A., Das, S., Arif, N., Sarraf, M., Zahra, N., Yadav, V., Aliniaeifard, S., Chauhan, D. K., and Hasanuzzaman, M., (2022). Diverse physiological roles of flavonoids in plant environmental stress responses and tolerance. *Plants,* 11(3158).

Singleton, V. L., Orthofer, R., and Lamuela-Raventós, R. M., (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. In L. Packer (Ed.), *Methods in Enzymology* (Vol. 299, pp. 152-178). San Diego, CA: Academic Press.

Stanković, M. S., Zia-Ul-Haq, M., Bojović, B. M., and Topuzović, M., (2014). Total phenolics, flavonoid content, and antioxidant power of *Cornus mas* L. *Bulgarian Journal of Agricultural Science,* 20(2), 358–363.

Wagenitz, G., (1975). *Centaurea* L. In P. H. Davis (Ed.), *Flora of Turkey and the East Aegean Islands* (Vol. 5, pp. 465–585). Edinburgh University Press.

Wink, M., (2003). Evolution of secondary metabolites from an ecological and molecular phylogenetic perspective. *Phytochemistry*, 64(1), 3-19.

Zengin, G., Aktumsek, A., Guler, G. O., Cakmak, Y. S., and Yildiztugay, E., (2011). Antioxidant properties of methanolic extract and fatty acid composition of *Centaurea urvillei* DC. subsp. *hayekiana* Wagenitz. *Records of Natural Products*, *5*(2).

Zhang, Z., Yang, M. J., and Pawliszyn, J., (1994). Solid-phase microextraction. A solvent-free alternative for sample preparation. *Analytical Chemistry*, 66(17), 844A–853A.