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ARAŞTIRMA MAKALESİ RESEARCH PAPER

Quantitative Profiling of Phenolic Content of *Carduus tenuiflorus* Curt. Extracts Based on LC–HRMS

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Abstract: Phenolic compounds, as plant-derived secondary metabolites, are of significant interest in food and pharmaceutical research due to their antioxidant potential. While the Carduus genus is known for its phenolic richness, comprehensive quantitative data on Carduus tenuiflorus Curt. remains limited. In this study, the phytochemical profile of C. tenuiflorus extracts was investigated using liquid chromatography–high-resolution mass spectrometry (LC–HRMS). The analysis enabled the reliable identification and quantification of 25 phenolic compounds, primarily consisting of phenolic acids and flavonoids. Among the phenolic acids, chlorogenic acid was found to be the most abundant, followed by salicylic, quinic, and p-coumaric acids. Within the flavonoid fraction, diosmetin, luteolin, apigenin, and their corresponding glycoside and glucuronide derivatives were predominant. This phytochemical profile aligns with the phenylpropanoid-dominated composition previously reported for other Carduus species. These findings provide quantitative evidence that C. tenuiflorus possesses a rich profile of phenolic acids and flavonoids, contributing baseline data for future investigations into its bioactive properties. As biological activities were not experimentally assessed within this scope, further research incorporating functional and biological evaluations is required to fully understand the species' therapeutic potential.

Keywords: Carduus tenuiflorus; flavonoids; LC–HRMS; phenolic compounds; phytochemical profile.

LC–HRMS ile *Carduus tenuiflorus* Curt. Ekstraktlarının Fenolik İçeriğinin Kantitatif Profillenmesi

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Öz: Fenolik bileşikler, bitki kökenli sekonder metabolitler olarak antioksidan potansiyelleri nedeniyle gıda ve farmasötik araştırmalarında büyük ilgi görmektedir. *Carduus* cinsinin fenolik bileşikler açısından zengin olduğu bilinmekle birlikte, *Carduus tenuiflorus* Curt. üzerine kapsamlı kantitatif veriler oldukça sınırlıdır. Bu çalışmada, *C. tenuiflorus* ekstraktlarının fitokimyasal profili sıvı kromatografisi–yüksek çözünürlüklü kütle spektrometrisi (LC–HRMS) kullanılarak araştırılmıştır. Yapılan analizler sonucunda, başlıca fenolik asitler ve flavonoidlerden oluşan 25 fenolik bileşik güvenilir bir şekilde tanımlanmış ve kantitatif olarak belirlenmiştir. Fenolik asitler arasında en yüksek miktarda bulunan bileşiğin klorojenik asit olduğu, bunu sırasıyla salisilik asit, kinik asit ve p-kumarik asidin izlediği belirlenmiştir. Flavonoid fraksiyonunda ise diosmetin, luteolin, apigenin ve bunların ilgili glikozit ve glukuronid türevlerinin baskın olduğu görülmüştür. Elde edilen bu fitokimyasal profil, *Carduus* türleri için daha önce bildirilen fenilpropanoid ağırlıklı bileşim ile uyumludur. Bu bulgular, *C. tenuiflorus*'un fenolik asitler ve flavonoidler bakımından zengin bir profile sahip olduğunu kantitatif olarak ortaya koymakta ve türün biyoaktif özelliklerine yönelik gelecekte yapılacak çalışmalar için temel veriler sunmaktadır. Bu çalışma kapsamında biyolojik aktiviteler deneysel olarak değerlendirilmemiştir; bu nedenle türün terapötik potansiyelinin tam olarak anlaşılabilmesi için fonksiyonel ve biyolojik değerlendirmeleri içeren ileri araştırmalara ihtiyaç duyulmaktadır.

Anahtar Kelimeler: *Carduus tenuiflorus*; LC–HRMS; fenolik bileşikler; fitokimyasal profil; flavonoidler.

INTRODUCTION

Phenolic compounds are important bioactive constituents widely distributed in plants and classified as secondary metabolites. Their characteristic aromatic structures bearing one or more hydroxyl groups confer notable antioxidant properties (Tungmunnithum et al., 2018; A. Singh et al., 2022; Öğretmen, 2022; Yolbaş, 2024b, 2024a, 2025; Beyatli, 2025). Although phenolic compounds are not essential for primary plant metabolism, they play crucial roles in plant defense responses and interactions with environmental stressors (Khadem & Marles, 2010). From a human health perspective, phenolic compounds have been associated with potential protective

effects against diseases linked to oxidative stress (Manach et al., 2004).

The antioxidant mechanisms of phenolic compounds include free radical scavenging, metal ion chelation, and inhibition of lipid peroxidation (Babbar et al., 2015; Balasundram et al., 2006). Oxidative stress has been implicated in the development of chronic disorders such as cancer, cardiovascular diseases, diabetes, and neurodegenerative conditions through damage to DNA, proteins, and lipids (Matés & Sánchez-Jiménez, 2000). Accordingly, dietary intake of plant-derived antioxidants is considered a complementary strategy for mitigating oxidative damage (Nehir El & Karakaya, 2004).

Medicinal and aromatic plants represent prominent natural sources of antioxidants due to their high phenolic content (Akbari et al., 2022; Deveci et al., 2023). Phenolic compounds have also been reported to suppress the formation of potential carcinogens such as heterocyclic aromatic amines and acrylamide (Zhu et al., 2009; Borrelli et al., 2002). Additionally, phenolic acids may contribute to cellular protection by preserving membrane integrity and may play supportive roles in metabolic disorders including obesity and insulin resistance (Alan & Kurşat, 2022).

Growing concerns regarding the long-term safety of synthetic antioxidants have increased interest in naturally occurring bioactive compounds (Yavaşer, 2011) . Consequently, analytical studies focusing on the identification and quantitative determination of plant-derived phenolics have gained importance (Jin et al., 2011). Medicinal plants have historically been used for nutritional and therapeutic purposes, and their relevance in the food, pharmaceutical, and cosmetic industries continues to expand (Göktaş & Gıdık, 2019; Oğan & Cömert, 2022).

Carduus tenuiflorus Curt. (slenderflower thistle) is a spiny winter annual species widely distributed in Mediterranean climates. It exhibits high ecological competitiveness, with seeds capable of germinating across a broad temperature range without dormancy. The presence of dense mucilage on the seed surface facilitates adaptation to diverse soil conditions and promotes rapid spread in disturbed habitats and pastures (Evans et al., 1979). This adaptability suggests that the bioactive components *of C. tenuiflorus*, particularly phenolic compounds, have the potential to provide health benefits.

The aim of this study is to conduct a comprehensive evaluation of the quality and quantity of phenolic compounds in *C. tenuiflorus*, and to determine the potential health benefits of this plant. The research also seeks to emphasize the importance of plant-based phenolic compounds in the fields of health and nutrition, while providing insight into future research areas.

MATERIAL AND METHOD

Collection of Plant Material: *Carduus tenuiflorus* samples were collected in May 2024 from Siirt Province, Turkey (37°55′00.6″N, 41°56′03.8″E). The aerial parts of the plants were dried at ambient temperature (approximately 24 °C) in the absence of direct sunlight. The dried material was subsequently ground into a homogeneous powder prior to analysis.

LC–HRMS Analysis

Sample Preparation: Ten milligrams of powdered *C. tenuiflorus* sample were extracted with a methanol–ultrapure water mixture (1:1, v/v). The extract was filtered through a 0.22 µm PTFE syringe filter and transferred into 1.5 mL vials for LC–HRMS analysis.

Chromatographic and Mass Spectrometric Conditions: Chromatographic separation was carried out using a Phenomenex Gemini NX-C18 column (3 µm, 110 Å; 100 × 2 mm) maintained at 30 °C. The mobile phase consisted of (A) ultrapure water containing 2% (v/v) glacial acetic acid and (B) LC–MS grade methanol. Elution was performed at a flow rate of 0.3 mL/min with an injection volume of 20 µL and a total run time of 20 min.

Mass spectrometric detection was performed using an Orbitrap Exactive Plus™ high-resolution mass spectrometer equipped with a heated electrospray ionization (HESI) source. Analyses were conducted in both positive and negative ionization modes using full-scan and all-ion fragmentation (AIF) acquisition. Instrumental parameters were set as follows: AGC target 3×10^6, spray voltage 3.5 kV, S-lens RF level 50, maximum injection time 2 ms, capillary temperature 350 °C, auxiliary gas temperature 350 °C, scan range 60–800 m/z, resolution 17,500, and collision energy <25 V.

Instrument Calibration and Data Processing: LC-HRMS analyses were carried out on a DIONEX UltiMate 3000RS platform (Thermo Fisher Scientific, Waltham, MA, USA) equipped with an autosampler, solvent delivery system, and column oven. Mass spectrometric detection was achieved using an Exactive Plus Orbitrap instrument operated with software version 2.1.0.I140 (Thermo Fisher Scientific). The mass calibration of the Orbitrap LC-MS system was performed using Pierce™ Negative Ion Calibration Solution and Pierce™ LTQ Velos ESI Positive Ion Calibration Solution to maintain mass accuracy. Throughout the analyses, chromatographic and mass spectrometric operations were controlled concurrently via TraceFinder 3.2 (Thermo Fisher Scientific). Acquisition and storage of analytical data were conducted using Xcalibur software version 2.1.0.I140 (Thermo Fisher Scientific).

RESULTS AND DISCUSSION

LC–HRMS analysis enabled the reliable identification and quantitative determination of 25 compounds exhibiting adequate chromatographic separation and reproducible signal intensity (table1). The majority of the identified compounds belonged to phenolic acids, flavonoids, and flavonoid glycosides. This phytochemical distribution is consistent with previous reports describing *Carduus* species as phenolic-rich taxa and aligns with general phytochemical patterns documented in the literature (Cardona et al., 1992; Marengo et al., 2017; Kozyra et al., 2019).

Among the phenolic acids, chlorogenic acid was by far the most abundant compound (46654.83 ng/g), followed by salicylic acid (3111.26 ng/g), quinic acid (1443.03 ng/g), *p*-coumaric acid (844.11 ng/g), ferulic acid (682.67 ng/g), and caffeic acid (660.82 ng/g). These findings are consistent with previous studies identifying chlorogenic acid as a major phenolic constituent in *Carduus* species.

Ferulic and caffeic acids were detected at moderate levels and represent typical products of the phenylpropanoid pathway. While their antioxidant and cytoprotective properties are well documented in the literatüre (Touaibia et al., 2011; Damasceno et al., 2017; Jo et al., 2019), such biological activities were not evaluated in the present study. The detection of quinic acid is noteworthy in the context of hydroxycinnamic acid biosynthesis and aligns with previous reports on the biological relevance of quinic acid derivatives (Zanello et al., 2015), although these effects were not experimentally verified here.

Salicylic acid was detected at relatively high levels. Although salicylic acid is known as the main metabolite of acetylsalicylic acid and has recognized pharmacological relevance (Stanley & Hegedus, 2000), the bioavailability and physiological implications of plant-derived free salicylic acid were beyond the scope of this study. Similarly, the presence of *p*-coumaric acid may indicate active flavonoid biosynthesis; however, no direct assessment of biosynthetic pathways was conducted (Tungmunnithum et al., 2018).

Several aromatic phenolics, including 3,4-dihydroxybenzaldehyde and vanillin, were detected at measurable levels. Although various biological activities have been attributed to these compounds (Ponnusamy et al., 2009; Bezerra et al., 2016; Li et al., 2018), no functional claims can be drawn from the present findings.

The flavonoid profile was characterized by the presence of diosmetin, luteolin, apigenin, and their glycoside and glucuronide derivatives. While the antioxidant and anti-inflammatory activities of these flavonoids are well documented (Middleton Jr et al., 2000; Seelinger et al., 2008; Ma et al., 2018), such activities were not experimentally assessed in this study.

Furthermore, the detection of compounds such as epigallocatechin gallate, orientin, rutin hydrate, quercetin, and kaempferol highlights the chemically diverse and multi-component nature of the *C. tenuiflorus* phenolic profile. Although biological activities of these compounds have been reported elsewhere (Rogerio et al., 2007; B. N. Singh et al., 2011; Ren et al., 2019; Baraka et al., 2022), the present study does not aim to validate these effects.

Overall, this study provides a detailed LC–HRMS-based phytochemical characterization of *Carduus tenuiflorus*, quantitatively demonstrating its richness in phenolic acids and flavonoids. The primary contribution of this work lies in the comprehensive quantitative profiling of a broad range of phenolic compounds using a single analytical platform. Nevertheless, the absence of direct biological activity assessment and the potential limitations in quantitative accuracy for certain compounds represent the main constraints of the study. Future investigations incorporating targeted bioassays and alternative extraction strategies will be essential for a more complete evaluation of the functional and pharmaceutical potential of *C. tenuiflorus*.

Table 1. Quantitative profile of phenolic compounds identified by LC-HRMS analysis.

No	Target Compound	Compound Type	Result (ng/g plant)
1	2,4-dihydroxybenzoic acid	Phenolic acid	N.D.
2	3-(4-Hydroxyphenyl) propionic acid	Phenolic acid	N.D.
3	3,4-dihydroxybenzaldehyde	Phenolic aldehyde	406.03
4	3,4-Dihydroxyphenylacetic acid (DOPAC)	Phenolic acid	N.D.
5	3-hydroxybenzoic acid (3-HBA)	Phenolic acid	N.D.
6	3-hydroxyphenylacetic acid (3-HPA)	Phenolic acid	N.D.
7	4-Hydroxybenzoic acid	Phenolic acid	N.D.
8	α-Cyano-4-hydroxycinnamic acid	Phenolic acid derivative	N.D.
9	Afzelin	Flavonoid glycoside	1157.86
10	Apigenin	Flavonoid aglycone	1396.36
11	Apigenin 7-glucoside	Flavonoid glycoside	N.D.
12	Apigenin 7-glucuronide	Flavonoid glycoside	1621.83
13	Apiin	Flavonoid glycoside	N.D.
14	Arbutin	Glycoside	N.D.
15	Astragalin	Flavonoid glycoside	N.D.
16	Benzoic acid	Phenolic acid	N.D.
17	Caffeic acid	Phenolic acid	660.82
18	Caffeic acid phenhyl ester (CAPE)	Phenolic ester	N.D.
19	Catechin (Cianidanol)-p	Flavan-3-ol	N.D.
20	Chlorogenic acid	Phenolic acid	46654.83
21	Chrysin	Flavonoid aglycone	N.D.
22	Coumaric acid	Phenolic acid	844.11
23	Daidzin	Isoflavone glycoside	N.D.
24	Diosmetin	Flavonoid aglycone	5694.11
25	Doxorubicin Hydrochloride	Anthracycline	N.D.
26	Ellagic acid	Polyphenol	N.D.
27	Emodin	Anthraquinone	7.38
28	Epigallocatechin	Flavan-3-ol	N.D.
29	Epigallocatechin gallate	Flavan-3-ol	251.25
30	Eriodictyol	Flavanone	N.D.
31	Esculin hydrate	Coumarin glycoside	N.D.
32	Ethylgallate	Phenolic ester	N.D.
33	Etoposide	Lignan derivative	539.14
34	Ferulic acid	Phenolic acid	682.67
35	Fisetin hydrate	Flavonoid	N.D.
36	Formononetin	Isoflavone	N.D.
37	Galangin	Flavonoid	N.D.
38	Gallic acid	Phenolic acid	N.D.
39	Genistein	Isoflavone	N.D.
40	Gentisic acid	Phenolic acid	N.D.
41	Glabridin	Isoflavonoid	N.D.
42	Hesperidin	Flavanone glycoside	N.D.
43	Homovanillic acid	Phenolic acid	N.D.
44	Isorhamnetin	Flavonoid aglycone	354.17
45	Isoquercitrin	Flavonoid glycoside	1343.58
46	Kaempferide	Flavonoid	N.D.
47	Kaempferitrin	Flavonoid glycoside	N.D.
48	Kaempferol	Flavonoid aglycone	346.67
49	Leucoside	Flavonoid glycoside	N.D.
50	Liquiritigenin	Flavanone	N.D.
51	Liquiritin	Flavanone glycoside	N.D.
52	Luteolin	Flavonoid aglycone	2289.83
53	Luteolin 7-rutinoside	Flavonoid glycoside	N.D.
54	Luteolin-7-O-glucuronide	Flavonoid glycoside	884.59
55	Luteoloside	Flavonoid glycoside	1357.47
56	Myricetin	Flavonoid aglycone	N.D.
57	Narcissin	Flavonoid glycoside	N.D.
58	Naringenin	Flavanone aglycone	57.24
59	Naringin	Flavanone glycoside	N.D.
60	Narirutin	Flavanone glycoside	N.D.
61	Neohesperidin	Flavanone glycoside	1287.16
62	Nicotiflorin	Flavonoid glycoside	N.D.

63	Oleuropein	Secoiridoid	N.D.
64	Orientin	Flavonoid C-glycoside	1447.97
65	Phloridzin	Chalcone glycoside	N.D.
66	Pinocembrin	Flavanone	N.D.
67	Protocatechuic acid	Phenolic acid	N.D.
68	Protocatechuic acid ethyl ester	Phenolic ester	N.D.
69	Quercetin	Flavonoid aglycone	N.D.
70	Quercetin 3-rutinoside 7-glucoside	Flavonoid glycoside	N.D.
71	Quinic acid	Alicyclic acid	1443.03
72	Rhoifolin	Flavonoid glycoside	N.D.
73	Rosmarinic acid	Phenolic acid	N.D.
74	Rutin hydrate	Flavonoid glycoside	382.71
75	Sakuranetin	Flavanone	N.D.
76	Salicylic acid	Phenolic acid	3111.26
77	Schaftoside	Flavonoid C-glycoside	N.D.
78	Sinapic acid	Phenolic acid	N.D.
79	Syringic acid	Phenolic acid	N.D.
80	Tiliroside	Flavonoid glycoside	N.D.
81	trans Cinnamic acid	Phenolic acid	N.D.
82	Vanillic acid	Phenolic acid	N.D.
83	Vanillin	Phenolic aldehyde	1015.93
84	Vicenin 2	Flavonoid C-glycoside	N.D.
85	Vitexin	Flavonoid C-glycoside	N.D.

N.D.: Not Detected

RESULT

The LC–HRMS-based quantitative analysis of *Carduus tenuiflorus* Curt. extracts enabled the reliable identification of 25 phenolic compounds, predominantly phenolic acids and flavonoids. Chlorogenic acid was the most abundant phenolic acid, while diosmetin, luteolin, apigenin, and their derivatives were prominent within the flavonoid fraction. These findings are generally consistent with previously reported phytochemical patterns in *Carduus* species and provide a foundational dataset for future functional and biological investigations of *C. tenuiflorus*.

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STATEMENTS AND DECLARATIONS

Author Contribution Statement: The author was solely responsible for the conception and design of the study, data analysis, interpretation of the results, and the writing and revision of the manuscript.

Conflicts of interest: The author declares that there is no conflict of interest regarding the publication of this paper.

Declaration of AI use: Limited AI-assisted tools were used for minor language and wording improvements during the preparation of the manuscript. The scientific content and all responsibility belong to the author.

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