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***Araştırma***
***(Research)***

# Comparative Analysis of Total Phenolic, Antioxidant Activity and Nutrient Element Composition of Fruits and Fruit Stalks of *Crataegus* spp. grown in Denizli (Türkiye)

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## Abstract

**Objective:** The aim of this study was to comparatively investigate the fruit and fruit stalks of *Crataegus monogyna* Jacq. and *Crataegus laevigata* (Poir.) DC collected from the Denizli/Türkiye region in terms of total phenolic content (TPC), DPPH radical scavenging activity, and macro and micro element compositions.

**Material and Methods:** Fruit and fruit stalk samples of *C. monogyna* and *C. laevigata* species were utilized. Methanolic extracts were derived from dried samples, and TPC (Folin-Ciocalteu method) and DPPH antioxidant activity were determined spectrophotometrically. Macro (P, K, Ca, Mg, S, Na) and micro (Fe, Cu, Mn, Zn, B) element concentrations were analyzed using an ICP-AES device. The obtained data were statistically evaluated by two-way analysis of variance and Box Plot analysis.

**Results:** The results indicated statistically significant differences ($p$<0.05) for most of the investigated parameters both between hawthorn species and between plant parts (fruit and stalk). Furthermore, significant interactions were detected between species and plant part. It was determined that TPC and DPPH activity were higher in fruit stalks compared to fruits. The highest TPC was measured in *C. monogyna* stalks (31.50 mg GAE/g DW), while the highest DPPH activity was observed in *C. laevigata* stalks (385.01 μmol TE/g DW). Macro and micro element contents also significantly varied depending on the species and plant part; P, Ca, Mg, S, Na, Fe, Cu, Mn, and Zn were generally found in higher concentrations in fruit stalks, whereas K content reached its highest value in *C. laevigata* fruit.

**Conclusion:** This study revealed that the fruits and particularly the fruit stalks of *C. monogyna* and *C. laevigata* are rich sources of bioactive compounds and minerals. Diversity among species and plant parts can be used to assess the nutraceutical and pharmaceutical potential of these species and can also be taken into account in breeding programs aimed at developing new hawthorn cultivars with targeted traits.

**Keywords:** *Crataegus monogyna*, *Crataegus laevigata*, Hawthorn, Macroelements, Microelements, Bioactive compounds

## Denizli (Türkiye)'de Yetişen *Crataegus* spp. Türlenine ait Meyve ve Meyve Saplarının Toplam Fenolik, Antioksidan Aktivite ve Besin Element Kompozisyonunun Karşılaştırmalı Analizi

## Öz

**Amaç:** Bu çalışmanın amacı, Denizli ilinden toplanan *Crataegus monogyna* Jacq. ve *Crataegus laevigata* (Poir.) alıç türlerinin meyve ve meyve saplarının toplam fenolik (TPC), DPPH radikal süpürme aktivitesi ve makro-mikro element kompozisyonları açısından karşılaştırmalı olarak incelenmesidir.

**Materyal ve Metot:** *C. monogyna* ve *C. laevigata* türlerine ait meyve ve meyve sapı örnekleri kullanılmıştır. Kurutulmuş örneklerden metanol özütleri hazırlanarak TPC (Folin-Ciocalteu metodu) ve DPPH antioksidan aktivitesi spektrofotometrik olarak belirlenmiştir. Makro (P, K, Ca, Mg, S, Na) ve mikro (Fe, Cu, Mn, Zn, B) element konsantrasyonları ise ICP-AES cihazı kullanılarak analiz edilmiştir. Elde edilen veriler, iki yönlü varyans analizi ve Box Plot analizi ile istatistiksel olarak değerlendirilmiştir.

**Bulgular:** Sonuçları incelenen çoğu parametre için hem alıç türleri arasında hem de meyve ve sap arasında istatistiksel olarak önemli farklar ($p$<0.05)

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tespit edilmiştir. Ayrıca, tür ve bitki kısmı arasında önemli etkileşimler saptanmıştır. TPC ve DPPH aktivitenin, meyvelere kıyasla meyve saplarında daha yüksek olduğu belirlenmiştir. En yüksek TPC *C. monogyna* sapında (31.50 mg GAE/g KM), en yüksek DPPH aktivitesi ise *C. laevigata* sapında (385.01 µmol TE/g KM) ölçülmüştür. Makro ve mikro element içerikleri de tür ve bitki kısmına göre önemli farklılıklar göstermiş; P, Ca, Mg, S, Na, Fe, Cu, Mn ve Zn genellikle meyve saplarında daha yüksek konsantrasyonlarda bulunurken, K içeriği *C. laevigata* meyvesinde en yüksek değere ulaşmıştır.

**Sonuç:** Bu çalışma, *C. monogyna* ve *C. laevigata* türlerinin meyve ve özellikle meyve saplarının zengin bir biyoaktif bileşik ve mineral kaynağı olduğunu ortaya koymuştur. Türler ve bitki kısımları arasındaki belirgin kimyasal çeşitlilik, bu türlerin besin ve ilaç potansiyellerinin değerlendirilmesinde ve hedeflenen özelliklere sahip yeni alıç çeşitlerinin geliştirilmesine yönelik ıslah programlarında dikkate alınması gerektiği ifade edilebilir.

**Anahtar kelimeler:** *Crataegus monogyna*, *Crataegus laevigata*, Alıç, Makro elementler, Mikro elementler, Biyoaktif bileşikler

## Introduction

Throughout history, fruits have played a critical role for humanity, not only as a food source but also in meeting fundamental needs such as health (Serçeoğlu and Önalan, 2024). Currently, there is a growing interest in plant-based products, particularly those classified as functional foods, which contain natural compounds with positive effects on health (Salık and Çakmakçı, 2023). Foremost among these compounds are substances that have antioxidant activities, which can neutralize the detrimental effects of free radicals formed as a result of metabolic processes in the body or due to environmental factors (Kor et al., 2018; Gümüştepe et al., 2022). Substances with antioxidant effects protect cells against damage caused by free radicals by reducing oxidative stress (Pandey and Rizvi, 2009) and may play a role in the prevention of many chronic diseases such as cancer and cardiovascular diseases (Fratta Pasini and Cominacini, 2023).

Fruits are rich sources of antioxidant activity, especially in terms of phenolic compounds (flavonoids, phenolic acids, etc.) (Mertoğlu and Evrenosoğlu, 2019; Kirca, 2025; Kırca et al., 2023; Kaya et al., 2024). The quantity and variety of phenolic compounds are important factors determining the antioxidant capacity of a plant (Prior et al., 2005). Radical scavenging methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl) are widely used to determine antioxidant activity (Thaipong et al., 2006; Kim et al., 2009; Özderin, 2024).

Hawthorn (*Crataegus* spp.), belonging to the *Rosaceae*, is widely distributed in the temperate regions of the Northern Hemisphere (Phipps et al., 2003) and comprises over 240 species worldwide (Özderin and Fakir, 2015; İkinci et al., 2022, Çalışkan et al., 2025). The leaves, flowers, and fruits of *Crataegus* species have been used in folk medicine for centuries (Karadeniz, 2004; Zhang et al., 2022). Preparations of leaves and flowers (sometimes as herbal tea) have traditionally been used in the treatment of cardiovascular disorders such as heart failure and hypertension, as well as conditions like diarrhea, insomnia, asthma, and inflammation, making it a medicinal fruit (Karadeniz, 2004; Karadeniz and Kalkışım, 2016; Lakache et al., 2014; Özderin et al., 2016; Lund et al., 2017). The German Commission E has approved a standardized leaf and flower extract for treating heart failure (Sticher and Meier, 1997), and the European Medicines Agency (EMA) also recognizes the use of hawthorn leaf and flower as a herbal medicinal product (European Medicines Agency, 2016). These health benefits attributed to hawthorn species are thought to be associated with the plant's rich profile of secondary metabolites, such as phenolic compounds, terpenes, aldehydes, and organic acids (Keser et al., 2014; Alirezalu et al., 2018). In particular, polyphenolic compounds like flavonoids (vitexin, rutin, hyperoside, quercetin), proanthocyanidins, and phenolic acids are known to be largely responsible for the observed cardiotonic, antioxidant, and anti-inflammatory effects of hawthorn (Barros et al., 2011; Edwards et al., 2012; Mraihi et al., 2013). Some studies indicate that hawthorn leaves can be a richer source of phenolic compounds compared to fruits and flowers (Keser et al., 2014; Alirezalu et al., 2018).

In addition to bioactive compounds, plants are an important source of macro and microelements essential for human nutrition and health (Çalışkan et al., 2018; Mertoğlu and Kırca, 2025). They contain high amounts of various mineral substances, primarily Ca, P, K, Mg, and Fe (Okatan et al., 2017). Mineral elements perform many fundamental functions in the body, from enzymatic reactions and bone health to nerve conduction and metabolic regulation (Welch and Graham, 2004). Calcium and phosphorus are fundamental components for bone mineralization and a healthy skeletal system, and are also necessary for cellular functions (Vannucci et al., 2018; Root, 2018). Furthermore, some minerals act as necessary co-factors for many biochemical reactions by contributing to the structural stability of enzymes (Dubey et al., 2020). Besides these, deficiency or excess of minerals can lead to adverse effects on human health, which is associated with a weakened immune system and an increased risk of exposure to various diseases (Merveille et al., 2024). Trace elements such as zinc and selenium are critically important for maintaining cellular integrity and the proper functioning of metabolism (Dubey et al., 2020; Sizova et al., 2024).

The flora of Türkiye possesses significant diversity in terms of the *Crataegus*, with numerous hawthorn species naturally occurring in the country (Davis, 1972; Dönmez, 2004), and the number of species may increase due to the formation of many different intermediate forms resulting from intraspecific and interspecific hybridization within populations (Caliskan et al., 2025). Among these species, *Crataegus monogyna* Jacq. and *Crataegus laevigata* (Poir.) DC. are commonly found (Christensen, 1992; Dönmez, 2004). However, findings regarding the chemical compositions (in terms of both bioactive compounds and mineral elements) of different hawthorn species, especially those growing under the same ecological conditions, and how this composition varies according to different plant parts (fruit, leaf, flower, stalk), are still insufficient. Although more studies exist on leaves and flowers (Keser et al., 2014; Alirezalu et al., 2018), detailed information about the potential bioactive and mineral content of fruit stalks, which emerge as a by-product during fruit processing and are often unutilized, is limited. There are many hawthorn populations in different regions of Turkey. Each region has hawthorn genotypes with their own characteristics. These areas should be studied and hawthorn genotypes with superior characteristics should be protected (Çolak and Alan, 2025).

This study aims to determine the total phenolic contents (TPC), antioxidant activities via DPPH radical scavenging, and essential macro (P, K, Ca, Mg, S, Na) and micro (Fe, Cu, Mn, Zn, B) element compositions of the fruit and fruit stalks of *C. laevigata* and *C. monogyna* species naturally growing in the Baklan region of Denizli province, thereby revealing the differences between species and plant parts, and the possible relationships among these parameters.

## Material and Methods

### Plant Material and Extraction Procedure

In this study, fruit and stalk of *Crataegus monogyna* Jacq. and *Crataegus laevigata* (Poir.) species obtained from the Baklan district of Denizli province (Türkiye) were used. During the last week of October, 20 fruit samples from each species were collected at harvest maturity. After the fruits were cleaned and separated from their stalks, they were oven-dried and stored at -20°C until analysis. Methanolic extracts obtained from the dried samples were used to determine antioxidant activity and total phenolic content. Macro and micro mineral contents were analyzed directly in dried and ground fruit samples.

The extraction process was initiated by adding 150 mL of methanol to 20 g of dried and ground fruit and stalk samples. The mixture was shaken at a speed of 200 rpm for 24 h. The resulting solution was filtered using coarse filter paper, and then the methanol was removed by evaporation at 40°C using a rotary evaporator. The powdered methanolic extract ultimately obtained was used for the analysis of total phenolic content and DPPH radical scavenging activity.

### Total Phenolic Content Analysis

Total phenolic content was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). For the analysis, 0.5 mL of diluted methanolic extracts was mixed with 2.5 mL of 0.2N Folin reagent, followed by the addition of 2 mL of saturated sodium carbonate ($Na_2CO_3$, 75 g/L). The mixture was incubated for 2 hours to complete the reaction, after which absorbance was measured at a wavelength of 765 nm using a spectrophotometer. The results obtained were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g DW).

### Antioxidant Capacity Analyses

The antioxidant capacity of the methanolic extracts was determined by measuring their ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical. This analysis was based on the method of Brand-Williams et al. (1995), with some modifications in the sample preparation stages as described by Kahve et al. (2024). For the analysis, 0.1 mL of methanolic extract was mixed with 3.9 mL of DPPH solution ($6 \times 10^{-5}$ M) prepared in methanol. The mixture was incubated for 30 minutes at room temperature in a dark environment, after which absorbance values were measured at a wavelength of 517 nm using a spectrophotometer. The results were expressed as micromoles of Trolox equivalent per kilogram of dry weight (μmol TE/kg DW).

**Macro and Micro Mineral Analyses**

For the analysis of macro and micro minerals, approximately 0.2 g of dried and ground samples were treated with 15 mL of pure nitric acid ($HNO_3$) and approximately 2 mL of hydrogen peroxide ($H_2O_2$). This mixture was subjected to a digestion process at 200 °C (MARS 5 microwave oven). The solution obtained after the digestion was diluted to a specific volume with ultrapure water and then filtered. The concentrations of macro and microelements were determined using an Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES) (Skujins, 1998).

**Statistical Analysis**

A two-way analysis of variance (ANOVA) was applied ($p<0.05$) to determine the main effects of two different plant parts (fruit and fruit stalk) of *C. laevigata* and *C. monogyna* species and the interaction between these two factors on total phenolic content (TPC), DPPH antioxidant activity, and macro (P, K, Ca, Mg, S, Na) and micro (Fe, Cu, Mn, Zn, B) element concentrations. Box plot graphs were generated to visualize the differences between species and plant parts. All statistical analyses and graph plotting were performed using RStudio software (R Core Team, 2024).

**Results and Discussion**

The differences in total phenolic content (TPC), DPPH antioxidant activity, macro (P, K, Ca, Mg, S, Na), and microelements (Fe, Cu, Mn, Zn, B) in the fruit and fruit stalk parts of *C. laevigata* and *C. monogyna* species, and the interactions of these factors are presented in Table 1. The analysis results show statistically significant differences for most of the investigated parameters, both between hawthorn species and between plant parts (Table 1). Furthermore, significant interactions were detected in the Species*plant parts for many of the parameters studied. These results are consistent with previous studies demonstrating significant differences in chemical composition both interspecifically and between different organs of the same plant within the *Crataegus* genus (Akbulut, 2024; Ravikumar et al., 2022). The significant effect of species, plant part, and the interaction of these two factors on most of the investigated parameters underscores the critical role these factors play in determining the biochemical and mineral profile of hawthorns. Such differences, in addition to genetic bases (Emami et al., 2018; Güney et al., 2018; Beigmohamadi et al., 2021; Christensen and Zieliński, 2008; Fichtner and Wissemann, 2021), can also be influenced by factors such as the environmental conditions in which the plant grows, harvest time, and the analytical methods used (Ravikumar et al., 2022). Significant interactions between species and plant parts suggest that inter-organ variations in one species may not be consistent in another. Therefore, each species–organ combination should be evaluated independently to avoid overgeneralization.

Regarding Total Phenolic Content (TPC), statistically significant main effects were found both between species ($p<0.01$) and between plant parts (fruit and stalk) ($p<0.001$). Additionally, a highly significant interaction was present between these two factors ($p<0.001$). When examining the interaction means, the highest TPC value was measured in *C. monogyna* stalk (31.50 mg GAE/g DW), which was statistically significantly higher than all other groups (*C. laevigata* stalk: 27.73 GAE/g DW, *C. laevigata* fruit: 10.56 GAE/g DW, *C. monogyna* fruit: 8.84 mg GAE/g DW). The second highest TPC was found in *C. laevigata* stalk (27.73 mg GAE/g DW), which was also significantly higher than the fruits of both species (10.56 and 8.84 mg GAE/g DW). When comparing fruits, *C. laevigata* fruit (10.56 mg GAE/g DW) contained significantly higher TPC than *C. monogyna* fruit (8.84 mg GAE/g DW). In both species, the TPC of stalks was significantly higher than that of fruits, although this difference between stalk and fruit was greater in *C. monogyna* species compared to *C. laevigata* species. The significantly higher TPC in fruit stalks compared to fruits in both species is consistent with general trends regarding the distribution of phenolic compounds in plants (Akbulut, 2024). These compounds can often be found in higher concentrations in vegetative parts that provide structural support or are more exposed to environmental stresses (UV radiation, pathogen attack) and may play a role in defense mechanisms (Taiz et al., 2015; Ravikumar et al., 2022). Akbulut (2024), in his study on *C. monogyna* and *C. laevigata*, found significant differences in TPC among different plant parts and reported that leaves and flowers generally had higher values than fruits. The significant differences observed between species and in the interaction indicate that the TPC profile is influenced by genetic (Fichtner and Wissemann, 2021) and possibly environmental factors. The highest TPC in *C. monogyna* stalk reveals that phenolic compound accumulation is particularly pronounced in the stalk tissue of this species. The higher TPC content in *C. laevigata* fruits compared to *C. monogyna* fruits parallels the findings of Akbulut (2024).

For DPPH antioxidant activity, significant main effects were detected between species ($p<0.01$) and plant parts ($p<0.001$), as well as a significant interaction ($p<0.05$). Examining the interaction means, the highest DPPH activity was measured in *C. laevigata* fruit stalk (385.01 μmol TE/g DW), which was statistically significantly higher than all other groups. The second highest activity was determined in *C. monogyna* fruit stalk (364.59 μmol TE/g DW), and this value was significantly different from *C. laevigata* stalk and the fruits of both species. The DPPH activities of the fruits were much lower; no statistically significant difference was found between *C. laevigata* fruit (25.01 μmol TE/g DW) and *C. monogyna* fruit (21.19 μmol TE/g DW). In both species, the antioxidant activity of fruit stalks was much higher than that of fruits, and the activity in stalks was significantly greater in *C. laevigata* species compared to *C. monogyna* species. DPPH activity, similar to TPC results, was found to be higher in fruit stalks compared to fruits. A similar trend was reported in the study by Akbulut (2024). This suggests that the high concentration of phenolic compounds in stalks directly reflects on the antioxidant capacity (Ravikumar et al., 2022). However, the finding in this study that *C. laevigata* stalk showed higher DPPH activity than *C. monogyna* stalk differs from the result reported by Akbulut (2024), where *C. monogyna* stalk had slightly higher activity. This difference may arise from variations in the geographical location where samples were collected, harvest time, or environmental stress factors (Ravikumar et al., 2022; Fichtner and Wissemann, 2021). The low DPPH activities of the fruits and the lack of significant difference between species indicate that the antioxidant capacity of fruits is more limited compared to stalks, but is at similar levels between species.

Regarding phosphorus (P) content, highly significant main effects were found for both species ($p<0.001$) and plant parts ($p<0.001$). However, the interaction between species and plant part was not statistically significant. Therefore, when examining the main effects, *C. laevigata* species (average 1821.25 mg/kg DW) generally had significantly higher P content than *C. monogyna* species (1675.00 mg/kg DW). Similarly, fruit stalks (1925.50 mg/kg DW) had significantly higher P content than fruits (1570.75 mg/kg DW). The higher phosphorus content in *C. laevigata* than in *C. monogyna*, and in stalks than in fruits, indicates that P accumulation differs between species and plant parts. The higher concentration of phosphorus in fruit stalks compared to fruits may be related to the mobility and distribution of P within the plant. P is a highly mobile element in plants and generally accumulates in metabolically active young tissues and reproductive organs (seeds, fruits) (Marschner, 2012). Akbulut (2024) reported that stalks contained higher P than fruit pulp, which supports the current finding. In a study on apples (Mertoğlu and Kırca, 2025), leaves were found to contain higher P than fruits. This is also consistent with the general trend that P can be found in higher concentrations in vegetative organs. The non-significant interaction indicates that the effects of species and plant part on P content are independent of each other.

For potassium (K) content, highly significant main effects were detected for species ($p<0.001$) and plant parts ($p<0.001$), as well as a highly significant interaction ($p<0.001$). Examining the interaction means, the highest K content was found in *C. laevigata* fruit (17393.00 mg/kg DW), and this value was statistically significantly higher than all other groups (*C. laevigata* stalk: 15865.50 mg/kg DW, *C. monogyna* fruit: 14184.50 mg/kg DW, *C. monogyna* stalk: 13746.00 mg/kg DW). *C. laevigata* stalk (15865.50 mg/kg DW) had the second highest value and was significantly higher than *C. monogyna* fruit (14184.50 mg/kg DW) and stalk (13746.00 mg/kg DW). Furthermore, *C. monogyna* fruit (14184.50 mg/kg DW) also contained significantly more K than its own stalk (13746.00 mg/kg DW). Therefore, while fruit K content in *C. laevigata* was significantly higher than in the stalk, *C. monogyna* fruit also contained more K than its stalk, but the difference was smaller. K is a very mobile macronutrient in plants and plays critical roles, especially during fruit development, such as osmotic regulation, sugar transport, and enzyme activation (Marschner, 2012; Mengel and Kirkby, 1987). Therefore, its accumulation in high concentrations in fruits is expected. The finding in this study that *C. laevigata* fruit had the highest K content contradicts the finding reported by Akbulut (2024) that stalks generally contained higher K than fruit pulp. This difference may be due to variations in the plant parts analyzed or environmental conditions. In a study on apples (Mertoğlu and Kırca, 2025), K was found at high levels in the fruit (ranging from 0.10%-0.94%, average 0.64%), but also in significant amounts in the leaves (1.66%-1.83%). This situation, where the distribution ratio of K, which is important for fruit development, between fruit and stalk differs according to species, may be due to its mobility within the plant.

Calcium (Ca) content was highly significantly affected by both species ($p<0.001$) and plant parts ($p<0.001$), and there was also a highly significant interaction between them ($p<0.001$). Examining the interaction means, the highest Ca content was measured in *C. monogyna* stalk (14228.00 mg/kg DW), and this value was statistically significantly higher than all other groups (*C. laevigata* stalk: 12661.50 mg/kg DW, *C. monogyna* fruit: 7387.50 mg/kg DW, *C. laevigata* fruit: 6313.50 mg/kg DW). *C. laevigata* stalk (12661.50 mg/kg DW) had the second highest Ca value and was significantly higher than the fruits of both species (7387.50 and 6313.50 mg/kg DW). When comparing fruits, *C. monogyna* fruit (7387.50 mg/kg DW) contained significantly more Ca than *C. laevigata* fruit (6313.50 mg/kg DW). In both species, stalks contained more Ca than fruits, but this difference was more pronounced in *C. monogyna*. The significantly higher calcium content in stalks compared to fruits in both species is consistent with Ca's low mobility within the plant and its general accumulation in structural tissues. Ca is primarily transported via the xylem and has very little mobility in the phloem, thus it tends to accumulate in tissues with high transpiration or older tissues such as leaves and stalks, while its transport to fruits is generally limited (Marschner, 2012; White and Broadley, 2003; Fichtner and Wissemann, 2021). The fact that Ca is an important component of cell wall structure (Taiz et al., 2015; Fichtner and Wissemann, 2021) can explain its high concentration in structural tissues like stalks. Akbulut (2024) also reported that stalks contained higher Ca than fruits. In apple (Mertoğlu and Kırca, 2025), the Ca concentration in leaves (1.49%-1.63%) was reported to be much higher than in fruit (0.04%-0.06%), which supports the tendency of Ca to accumulate in vegetative parts. The highest Ca value in *C. monogyna* stalk and its fruit also containing more Ca than *C. laevigata* fruit indicates differences in Ca uptake and distribution between species.

Although there was no significant main effect of species on magnesium (Mg) content, the plant part ($p<0.001$) and interaction ($p<0.001$) were highly significant. According to the interaction means, the highest Mg content was detected in *C. monogyna* stalk (2202.00 mg/kg DW), and this value was statistically significantly higher than all other groups (*C. laevigata* stalk: 1902.50 mg/kg DW, *C. laevigata* fruit: 1336.50 mg/kg DW, *C. monogyna* fruit: 1163.00 mg/kg DW). *C. laevigata* stalk (1902.50 mg/kg DW) had the second highest value and was significantly higher than the fruits of both species (1336.50 and 1163.00 mg/kg DW). Among the fruits, *C. laevigata* fruit (1336.50 mg/kg DW) contained significantly more Mg than *C. monogyna* fruit (1163.00 mg/kg DW). Although Mg is more mobile than Ca, it can still accumulate more in vegetative organs like leaves compared to fruits (Marschner, 2012). It was determined that stalks contained more Mg than fruits in both species. The higher concentration of magnesium in stalks compared to fruits, similar to calcium, is a finding also supported by Akbulut (2024). The non-significant main effect of species despite a significant interaction indicates that Mg distribution exhibits a complex pattern depending on species and plant part. The highest Mg value in *C. monogyna* stalk, while *C. laevigata* contained higher Mg when comparing fruits, reflects this complexity.

For sulfur (S) content, species ($p<0.05$), plant part ($p<0.001$), and interaction ($p<0.05$) yielded significant results. According to the interaction means, the highest S content was found in *C. monogyna* stalk (963.00 mg/kg DW), and this value was statistically significantly higher than *C. laevigata* stalk (877.00 mg/kg DW) and the fruits of both species (*C. laevigata* fruit: 632.50 mg/kg DW, *C. monogyna* fruit: 631.00 mg/kg DW). *C. laevigata* stalk (877.00 mg/kg DW) also had significantly higher S content than the fruits of both species (632.50 and 631.00 mg/kg DW). However, no statistically significant difference was found in S content between *C. laevigata* (632.50 mg/kg DW) and *C. monogyna* (631.00 mg/kg DW) fruits. Sulfur is necessary for plants to produce some vitamins (B group vitamins and biotin) (Bayrak and Yanardağ, 2021; Miret and Munné-Bosch, 2013). Vitamin B7 (biotin) is a sulfur-containing vitamin and acts as a coenzyme in carboxylation reactions of enzymes (Lachowicz et al., 2024). S is found in the structure of sulfur-containing amino acids (methionine, cysteine), proteins, and some secondary metabolites (Marschner, 2012; Narayan et al., 2022; Zhou et al., 2024). The higher sulfur content in stalks compared to fruits, like some other macroelements, is a trend also reported by Akbulut (2024). The highest S value in *C. monogyna* stalk indicates greater S accumulation in the stalk tissue of this species. The lack of a significant difference between species in fruits suggests that fruit S content may be at similar levels regardless of species.

Sodium (Na) content was significant affected by species ($p<0.001$), plant part ($p<0.001$), and their interaction ($p<0.001$). According to the interaction means, the highest Na content was measured in *C. laevigata* stalk (91.67 mg/kg DW), and this value was statistically significantly higher than *C. monogyna* stalk (79.32 mg/kg DW) and the fruits of both species (*C. laevigata* fruit: 32.83, *C. monogyna* fruit: 34.48 mg/kg DW). *C. monogyna* stalk (79.32 mg/kg DW) also had significantly higher Na content than the fruits of both species (32.83 and 34.48 mg/kg DW). However, no statistically significant difference was observed in Na content between *C. laevigata* (32.83 mg/kg DW) and *C. monogyna* (34.48 mg/kg DW) fruits. The significantly higher sodium in stalks compared to fruits and the highest value in *C. laevigata* stalk are consistent with the results reported by Akbulut (2024). Akbulut (2024) also found higher Na in stalks than in fruit pulp and reported higher Na in *C. laevigata* stalk than in *C. monogyna* stalk. The lack of difference between species in fruits is also consistent with Akbulut's (2024) findings. Sodium (Na) is generally not considered an essential element for plants, but it may have some physiological roles, especially under K deficiency or saline conditions (Marschner, 2012), and particularly when potassium is low, it can contribute to some physiological functions such as plant growth and stomatal behavior (Horie et al., 2007; Septiyana et al., 2019). Higher Na levels in stalks may be associated with uptake from the soil and accumulation after transport via the xylem.

Iron (Fe) content was significant affected by species ($p<0.001$), plant part ($p<0.001$), and their interactions ($p<0.001$). Examining the interaction means, the highest Fe content was detected in *C. laevigata* stalk (108.28 mg/kg DW), and this value was statistically significantly higher than all other groups (*C. monogyna* stalk: 101.15 mg/kg DW, *C. laevigata* fruit: 53.90 mg/kg DW, *C. monogyna* fruit: 22.90 mg/kg DW). *C. monogyna* stalk (101.15 mg/kg DW) had the second highest value and was significantly higher than the fruits of both species (53.90 and 22.90 mg/kg DW). When comparing fruits, *C. laevigata* fruit (53.90 mg/kg DW) contained significantly more Fe than *C. monogyna* fruit (22.90 mg/kg DW). In both species, stalks contained more Fe than fruits, and *C. laevigata* had higher Fe values than *C. monogyna* in both plant parts. The higher iron content in stalks than in fruits and in *C. laevigata* than in *C. monogyna* is generally consistent with the results reported by Akbulut (2024); Akbulut (2024) also found higher Fe in stalks than in fruits and reported that *C. laevigata* (in both stalk and fruit pulp) contained higher Fe than *C. monogyna*. Mertoğlu and Kırca (2025) reported that Fe concentration in leaves was significantly higher than in fruit. This indicates that Fe also tends to accumulate more in the vegetative parts of plants. The significant difference between species may indicate differences in genetic uptake and translocation capacities. Fe is an essential microelement for plants and plays a critical role in many redox reactions, such as chlorophyll synthesis, respiration, and electron transport chains in photosynthesis (Marschner, 2012; Mengel and Kirkby, 1987; Mordoğan et al., 2018; Akınoğlu and Korkmaz, 2021).

Copper (Cu) content was significant affected by species ($p<0.001$), plant part ($p<0.001$), and interaction ($p<0.01$). According to the interaction means, the highest Cu content was measured in *C. laevigata* stalk (12.47 mg/kg DW), and this value was statistically significantly higher than *C. monogyna* stalk (11.74 mg/kg DW) and the fruits of both species (*C. laevigata* fruit: 10.65 mg/kg DW, *C. monogyna* fruit: 8.41 mg/kg DW). *C. monogyna* stalk (11.74 mg/kg DW) also had significantly higher Cu content than the fruits of both species (10.65 and 8.41 mg/kg DW). Among the fruits, *C. laevigata* fruit (10.65 mg/kg DW) contained significantly more Cu than *C. monogyna* fruit (8.41 mg/kg DW). Copper distribution also shows a similar pattern to iron. Higher concentrations were found in stalks than in fruits, and among species, this was at a higher level in *C. laevigata* than in *C. monogyna*. Copper (Cu), like Fe, is an essential microelement involved in redox reactions and is found in the structure of important enzymes such as plastocyanin (photosynthesis), cytochrome c oxidase (respiration), and Cu/Zn-superoxide dismutase (antioxidant defense) (Liesivuori and Savolainen, 1991; Chan et al., 1998; Milne et al., 1999; Barceloux et al., 2002). Our findings are consistent with the results reported by Akbulut (2024), who stated that stalks contained higher Cu than fruit pulp, and *C. laevigata* contained higher Cu than *C. monogyna*. Similarly, Mertoğlu and Kırca (2025) reported that Cu content in leaves was higher than in fruit. The observed differences and the Cu uptake and accumulation abilities of the species may stem from genetic differences.

Regarding manganese (Mn) content, highly significant main effects were found for both species ($p<0.001$) and plant parts ($p<0.001$). However, the interaction between species and plant part was not statistically significant. In general, *C. laevigata* species (average 18.15 mg/kg DW) had significantly higher Mn content than *C. monogyna* species (average 15.90 mg/kg DW). Similarly, fruit stalks (average 21.58 mg/kg DW) had significantly higher Mn content than fruits (average 12.47 mg/kg DW). Manganese (Mn) is required for the photolysis of water in photosynthesis and the activity of the Mn-superoxide dismutase (Mn-SOD) enzyme (Sevilmiş et al., 2020). The Mn-SOD enzyme helps plants manage oxidative stress and generally increases plant resistance to stressful conditions (Ataş et al., 2017). The higher manganese content in *C. laevigata* than in *C. monogyna* and in stalks than in fruits is consistent with the results reported by Akbulut (2024); Akbulut (2024) also reported higher Mn levels in stalks than in fruit pulp and in *C. laevigata* than in *C. monogyna*. Apple leaves have also been reported to contain higher Mn than fruits (Mertoğlu and Kırca, 2025), which supports the tendency of Mn to accumulate in vegetative organs.

Zinc (Zn) content was significant affected by species ($p<0.001$), plant part ($p<0.001$), and interaction ($p<0.001$). Examining the interaction means, the highest Zn content was detected in *C. laevigata* stalk (14.20 mg/kg DW), and this value was statistically significantly higher than all other groups (*C. monogyna* stalk: 13.44 mg/kg DW, *C. laevigata* fruit: 10.74 mg/kg DW, *C. monogyna* fruit: 8.25 mg/kg DW). *C. monogyna* stalk (13.44 mg/kg DW) had the second highest value and was significantly higher than the fruits of both species (10.74 and 8.25 mg/kg DW). When comparing fruits, *C. laevigata* fruit (10.74 mg/kg DW) contained significantly more Zn than *C. monogyna* fruit (8.25 mg/kg DW). In both species, stalks contained more Zn than fruits, and *C. laevigata* had higher Zn values than *C. monogyna* in both plant parts. Zinc distribution, similar to iron and copper, shows higher concentrations in stalks than in fruits and in *C. laevigata* than in *C. monogyna*. This trend was also reported by Akbulut (2024). Mertoğlu and Kırca (2025) reported that Zn content in leaves was significantly higher than in fruit and that Zn levels in fruit could potentially be low. This indicates that Zn is also retained more in vegetative parts. Zinc is required for the activity of many enzymes and protein synthesis (Marschner, 2012; Han and Sönmez, 2019).

While there was a highly significant main effect of species on boron (B) content ($p<0.001$), there was no significant main effect of plant parts. However, there was a highly significant interaction between species and plant part ($p<0.001$). According to the interaction means, *C. laevigata* fruit (23.56 mg/kg DW) and stalk (22.71 mg/kg DW) had similar and the highest B content, and these two groups contained statistically significantly higher B than *C. monogyna* stalk (18.34 mg/kg DW) and fruit (16.69 mg/kg DW). Within the *C. monogyna* species, the stalk (18.34 mg/kg DW) had significantly higher B content than the fruit (16.69 mg/kg DW). Therefore, while B content was higher in the stalk than in the fruit in *C. monogyna* species, no significant difference was observed between fruit and stalk in *C. laevigata* species. Boron distribution exhibits a different pattern from other microelements. The non-significant main effect of plant part despite a highly significant interaction suggests that B distribution may be species-specific. Akbulut (2024) reported that for *C. monogyna*, the stalk contained higher B than the fruit pulp, which supports the current finding. Mertoğlu and Kırca (2025), however, reported that B concentration in fruit was slightly higher than in leaves. This indicates that B distribution can vary significantly according to plant species. Boron is important in processes such as cell wall structure and pollen germination, and its transport largely depends on the transpiration stream, which can affect its accumulation in different organs (Marschner, 2012; Mengel and Kirkby, 1987). The generally higher B content in *C. laevigata* than in *C. monogyna* suggests differences in B uptake or utilization efficiency between species. Box plot analysis shows that there are significant differences in biochemical parameters and macro and micro element contents in the fruits and fruit stalks of *C. monogyna* and *C. laevigata* species (Figure 1). Total phenolic content is approximately 3 times higher in the fruit stalks compared to the fruits in both species. While phenolic content is higher in *C. laevigata* fruits compared to *C. monogyna*, *C. monogyna* has higher phenolic content in its fruit stalks. DPPH antioxidant activity is dramatically higher (approximately 15 times) in fruit stalks compared to fruits, and the fruit stalks of *C. laevigata* show the highest antioxidant activity.

Table 1. Effects of Species (*C. laevigata* and *C. monogyna*) and Plant Part (Fruit and Fruit Stalk) on Total Phenolic Content (mg GAE/g DW), DPPH Activity (µmol TE/g DW), and Mineral Elements (mg/kg)

| **Species** | **TPC** | **DPPH** | **P** | **K** | **Ca** | **Mg** | **S** | **Na** | **Fe** | **Cu** | **Mn** | **Zn** | **B** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *C. laevigata* | 19,14 b | 205,01 a | 1821,2 a | 16629,25 a | 9487,50 b | 1619,50 a | 754,75 b | 63,07 a | 81,09 a | 11,56 a | 18,15 a | 12,47 a | 23,13 a |
| *C. monogyna* | 20,17 a | 192,89 b | 1675,0 b | 13965,25 b | 10807,75 a | 1682,50 a | 797,00 a | 56,07 b | 62,03 b | 10,07 b | 15,90 b | 10,85 b | 17,51 b |
| **Plant parts** | | | | | | | | | | | | | |
| Fruit | 9,70 b | 23,10 b | 1570,7 b | 15788,75 a | 6850,50 b | 1249,75 b | 637,75 b | 33,66 b | 38,40 b | 9,53 b | 12,47 b | 9,49 b | 20,12 a |
| Fruit stalk | 29,61 a | 374,80 a | 1925,5 a | 14805,75 b | 13444,75 a | 2052,25 a | 920,00 a | 85,49 a | 104,71 a | 12,10 a | 21,58 a | 13,82 a | 20,52 a |
| **Species*Plant parts** | | | | | | | | | | | | | |
| *C. laevigata* / Fruit | 10,56 c | 25,01 c | 1666,0 c | 17393,00 a | 6313,50 d | 1336,50 c | 632,50 c | 32,83 c | 53,90 c | 10,65 c | 13,50 c | 10,74 c | 23,56 a |
| *C. monogyna* / Fruit | 8,84 d | 21,19 c | 1475,5 d | 14184,50 c | 7387,50 c | 1163,00 d | 631,00 c | 34,48 c | 22,90 d | 8,41 d | 11,44 d | 8,25 d | 16,69 c |
| *C. laevigata* / Fruit stalk | 27,73 b | 385,01 a | 1976,5 a | 15865,50 b | 12661,50 b | 1902,50 b | 877,00 b | 91,67 a | 108,28 a | 12,47 a | 22,79 a | 14,20 a | 22,71 a |
| *C. monogyna* / Fruit stalk | 31,50 a | 364,59 b | 1874,5 b | 13746,00 d | 14228,00 a | 2202,00 a | 963,00 a | 79,32 b | 101,15 b | 11,74 b | 20,36 b | 13,44 b | 18,34 b |
| *Anova* | | | | | | | | | | | | | |
| $F_{Species}$ | 23,84** | 32,18** | 65,72** | 2068,09*** | 2531,91*** | 5,62 ns | 10,14* | 243,19*** | 1720,60*** | 78,23*** | 100,45*** | 119,22*** | 584,13*** |
| $F_{Plant\ parts}$ | 8954,3*** | 27111,04*** | 386,70*** | 281,58*** | 63163,52*** | 912,35*** | 471,92*** | 13335,16*** | 20828,51*** | 235,23*** | 1652,24*** | 849,77*** | 2,99 ns |
| $F_{Species*Plant\ parts}$ | 170,47*** | 15,09* | 6,02 ns | 86,40*** | 88,08*** | 79,24*** | 10,87* | 142,06*** | 674,65*** | 19,96** | 0,69 ns | 33,99*** | 28,76*** |

Significance levels are indicated by * p<0.05, ** p<0.01, *** p<0.001, while non-significant differences are indicated by 'ns'. Differences between means are indicated by letters (a, b, c, d). Means not sharing the same letter are statistically different.

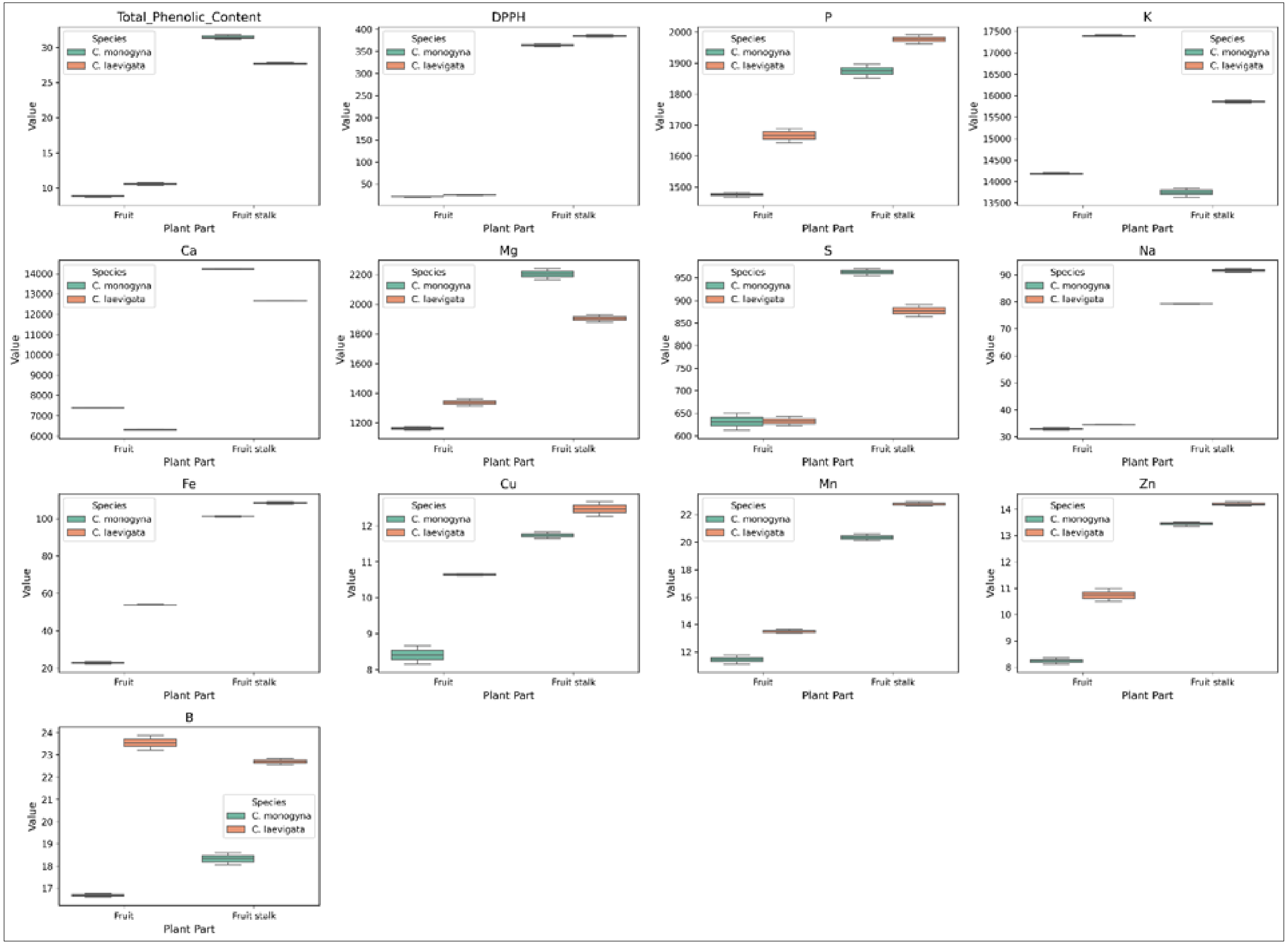


Figure 1. Comparative analysis of biochemical parameters and macro and micro element contents in the fruits and fruit stalks of *C. monogyna* and *C. laevigata* species

Looking at macroelements, phosphorus (P) concentration is higher in fruit stalks, with the fruit stalks of *C. laevigata* having the highest P values. While potassium (K) content is significantly higher in *C. laevigata* fruits, it is at its lowest level in the fruit stalks of *C. monogyna*. Calcium (Ca) is found in approximately 2 times higher concentrations in fruit stalks compared to fruits, and the fruit stalks of *C. monogyna* show the highest Ca values. Magnesium (Mg) content is also higher in fruit stalks, with the fruit stalks of *C. monogyna* having the highest Mg concentration. Sulfur (S) concentration is similarly higher in fruit stalks, and the fruit stalks of *C. monogyna* show the highest values.

When microelements are examined, sodium (Na) is approximately 2.5 times higher in fruit stalks compared to fruits, and the fruit stalks of *C. laevigata* have the highest Na content. Striking differences are observed in iron (Fe) concentrations, with approximately 2.5 times higher Fe in *C. laevigata* fruits compared to *C. monogyna* fruits. In fruit stalks, Fe content is much higher than in fruits. Copper (Cu) content is higher in fruit stalks, and the fruit stalks of *C. laevigata* show the highest Cu concentration. Manganese (Mn) concentration is approximately 2 times higher in fruit stalks compared to fruits, and the fruit stalks of *C. laevigata* have the highest Mn values. Zinc (Zn) content is also higher in fruit stalks, and the fruit stalks of both species show similar Zn concentrations. Boron (B) content reaches its highest values in *C. laevigata* fruits, while no significant difference is observed between species in fruit stalks.

Our results indicate that the fruits and fruit stalks of hawthorn species have different biochemical profiles and elemental compositions. The generally higher phenolic content, antioxidant activity, and elemental concentrations exhibited by fruit stalks suggest that these plant parts can be considered as potential sources of bioactive compounds. Furthermore, the differences between species reflect the diversity of genetic and metabolic pathways and suggest that these species may have different physiological and ecological adaptations.

*C. monogyna* is remarkably rich in phenolic compounds, which play a significant role in its antioxidant activity. Goudjil et al. (2024) highlight the phenolic compounds identified in *C. monogyna* using advanced techniques such as LC-ESI-MS/MS to analyze these components and emphasize their potent antioxidant activities. Variations in phenolic content due to genetic and environmental factors have been investigated, and the species has been reported as a valuable resource for nutraceutical applications (Tamayo-Vives et al., 2023). Similarly, Stoenescu et al. (2022) stated that the total phenolic content obtained from different species shows antioxidant benefits that can vary significantly depending on extraction methods. Studies have emphasized significant variations in antioxidant activity among different plant organs and genotypes, with antioxidant activity measured by the FRAP method varying considerably (Alirezalu et al., 2020; Alirezalu et al., 2018). Radi et al. (2023) confirmed the presence of various bioactive compounds as well as significant antioxidant activities in *C. monogyna*, thereby underlining the potential applications of these extracts in combating chronic diseases, including diabetes and cardiovascular complications. Furthermore, Amrati et al. (2024) highlighted the anti-inflammatory and hepatoprotective effects of *C. monogyna*, particularly noting the presence of compounds such as chlorogenic acid and rutin, supporting its pharmacological importance.

The biochemical diversity revealed in our study, and particularly the rich phytochemical profile of *C. monogyna*, is consistent with literature findings supporting the broad pharmacological potential of this species. Yağlıoğlu et al. (2015) showed that various plant extracts, including *C. monogyna*, exhibited antiproliferative activities on cell lines, indicating the anticancer potential of the species. Similarly, Meravanige et al. (2023) discussed the therapeutic effects of *C. monogyna* inhibitors against breast cancer, emphasizing its potential in cancer treatment. Amrati et al. (2024) further reinforced its pharmacological importance by evaluating the anti-inflammatory, hepatoprotective, and antileukemic effects of *C. monogyna* extract. Such bioactivities may be closely related to the high phenolic content and antioxidant activity observed in our study. In this context, breeding programs aimed at enhancing the medicinal properties of hawthorn species are of great importance. Goudjil et al. (2024), by analyzing bioactive compounds in Algerian *C. monogyna*, stated that improved cultivars through selective breeding for higher phenolic content could enhance medicinal properties. Alirezalu et al. (2018) also reported that the flavonoid profiles and antioxidant activities in different *Crataegus* species showed that breeding efforts could focus on improving specific traits. Furthermore, the methods for optimizing phenolic extraction by Martín-García et al. (2021) and the study on the pharmacological properties of hawthorn by Martinelli et al. (2021) are supportive of breeding strategies for improved health benefits. Therefore, the phytochemical and elemental diversity identified in this study provides fundamental data for the targeted breeding of *Crataegus* species and the full assessment of their nutraceutical/pharmaceutical potential.

## Conclusion

This study revealed that the fruits and fruit stalks of *C. monogyna* and *C. laevigata* species grown in the Denizli region exhibit significant differences in terms of total phenolic content, DPPH antioxidant activity, and macro/microelement compositions. Statistical analyses showed significant variations for most of the investigated parameters both between hawthorn species and between plant parts (fruit and stalk), and significant interactions were found between these two factors. In general, fruit stalks were determined to have markedly higher total phenolic content and DPPH antioxidant activity compared to fruits. Specifically, *C. monogyna* stalk had the highest total phenolic content, while *C. laevigata* stalk possessed the highest DPPH activity. Macro and microelement concentrations also showed significant changes depending on the species and plant part, with fruit stalks generally exhibiting higher mineral content than fruits. While Ca, Mg, S, Na, Fe, Cu, Mn, and Zn concentrations were found to be higher in fruit stalks, K content reached its highest value in *C. laevigata* fruit. These findings indicate that hawthorn species, and particularly their fruit stalks, are a rich source of bioactive compounds and minerals, but these contents can vary significantly according to species and plant part. Therefore, considering this diversity is of great importance in evaluating the nutraceutical and pharmaceutical potential of these species. More importantly, this identified chemical diversity offers a valuable genetic resource and selection criterion for future hawthorn breeding programs; thereby, it may be possible to develop new hawthorn cultivars with superior characteristics in terms of targeted bioactive compounds or specific mineral profiles and to optimize the use of these plants in the fields of functional food and phytotherapy.

## Conflict of Interest

There is no conflict of interest among the authors.

## Author Contributions Statement

LK: Conceptualization, Methodology, Investigation, Statistical analysis and Data visualization, Writing, Review and Editing. TB: Methodology, Analysis, Writing, Review and Editing. The authors read and approved the final manuscript.

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