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Phenolic Profile and Antioxidant Potential of Hawthorn From The Sivas Region of Türkiye

Sivas/ Türkiye Alıcının Fenolik Profili ve Antioksidan Potansiyeli

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Abstract

Hawthorn (*Crataegus spp*.) fruits are widely recognized for their biologically active components and high nutritional content. This study examined the total flavonoid content (TFC), the total phenolic content (TPC), the antioxidant activity, and the phenolic composition of hawthorn fruit obtained from Sivas markets in Türkiye. The TPC and TFC contents were determined to be 2.160 mg GAE/ g FW and 0.193 mg QE/ g FW, respectively. Antioxidant activity was assessed using FRAP and DPPH assays with values of 0.709±0.028 µmol TE/g FW and 3.563±0.039 mg/mL, respectively. According to findings of phenolic composition, *p*-OH benzoic acid and ellagic acid were found to be the most abundant phenolic components of hawthorn fruits. In particular, this study revealed the existence of significant phenolic compounds pinocembrin (0.176 µg/g FW) and chrysin (0.166 µg/g FW) in hawthorn fruits for the first time, developing the understanding of variation in phytochemical composition and antioxidant potential. The outcomes of this research will provide important information about the phenolic composition, antioxidant activity, and potential nutritional benefits of hawthorn fruits from this area.

Keywords: Hawthorn, Antioxidant activity, Phenolic composition, Sivas.

Özet

Alıç (*Crataegus spp*.) meyvesi, biyolojik olarak aktif bileşenleri ve yüksek besin içeriği ile geniş çapta tanınmaktadır. Bu çalışmada, Türkiye'nin Sivas pazarlarından temin edilen alıç meyvesinin toplam flavonoid içeriği (TFC), toplam fenolik içeriği (TPC), antioksidan aktivitesi ve fenolik bileşimini incelenmiştir. TPC ve TFC değerleri sırasıyla 2.160 mg GAE/g FW ve 0.193 mg QE/g FW olarak belirlenmiştir. Antioksidan aktivite, FRAP ve DPPH yöntemleri kullanılarak değerlendirilmiş olup sırasıyla 0.709±0.028 µmol TE/g FW ve 3.563±0.039 mg/mL olarak ölçülmüştür. Fenolik bileşim bulgularına göre, alıç meyvesinin en yüksek fenolik bileşenleri olarak p-OH benzoik asit ve ellajik asit tespit edilmiştir. Özellikle bu çalışma, alıç meyvesinde ilk kez önemli fenolik bileşikler olan pinosembrin (0.176 µg/g FW) ve kersin (0.166 µg/g FW) varlığını ortaya koyarak fitokimyasal bileşimdeki çeşitlilik ve antioksidan potansiyelin anlaşılmasını geliştirmiştir. Bu araştırmanın sonuçları, bölgedeki alıç meyvelerinin fenolik bileşimi, antioksidan aktivitesi ve potansiyel besin faydaları hakkında önemli bilgiler sağlayacaktır.

Anahtar Kelimeler: Alıç, Antioksidan aktivite, Fenolik bileşimi, Sivas.

1. INTRODUCTION

This Hawthorn, a member of the *Crataegus* genus of the Rosaceae family, is one of the widespread fruit species in Asia, Central America, and areas bordering the Mediterranean, including sections of Europe and North Africa. Berries and bioactive components of hawthorn have been demonstrated to have a variety of beneficial features particularly antibacterial, anti-inflammatory, intestinal and cardiovascular protection, neuroprotective, and anti-diabetic effects in recent research. It is estimated that there are approximately 240 different species of hawthorn in the world. Among these, *Crataegus monogyna* stands out for its wide distribution and extensive use as a medicinal herb (Oğuzhan Çalışkan, 2022). Although *Crataegus monogyna* is distributed almost everywhere in Türkiye, *Crataegus aronia*, *Crataegus orientalis*, and *Crataegus oxyacantha* are also common species (Sümbül *et al.*, 2024; Oğuzhan Çalışkan, 2022). In addition, it's been estimated that about 30 distinct hawthorn species have been identified in Anatolia to date.

In Türkiye, although hawthorn is commonly grown in Hatay, regions such as Gümüşhane, Tokat, Bolu, and Sivas are known for harvesting the fruit from nature for food. Hawthorn's health benefits and increasing demand for large-fruited hawthorns as well as high total phenolic content, total soluble solids, antioxidant capacity, and antioxidant activity in Hatay, province eastern Mediterranean region of Türkiye suggest that this type will remain popular in the future (Oğuzhan Çalışkan, 2022). Therefore, broadening hawthorn cultivation throughout the country is important to meet this increasing demand. Despite recent efforts by producers and researchers to develop exceptional hawthorn genotypes through selection works

and grafting techniques, a large population of hawthorn trees in Türkiye remains underexplored. Additionally, identifying phenolic components and the antioxidant capacity of local species of hawthorn genotypes may lead to the inclusion or valorization of these species as potential nutraceuticals (González-Jiménez *et al.*, 2018).

With its desirable soil and climate conditions, Sivas is well-known for its natural hawthorn species and has a significant potential for hawthorn cultivation (Sümbül *et al.*, 2024; Ağlar, 2020). Natural hawthorn fruit species that often grow in Sivas's hilly regions, attract the attention of enthusiasts in autumn. Depending on preference, hawthorn fruits harvested from mountain trees are consumed or made into marmalade and vinegar. Those who trade bring the hawthorns they collect from the mountains to consumers in the city markets. Thus, we examined the antioxidant activity and the phenolic profile of hawthorn species in Sivas to realize this potential and meet the increasing demand of the market.

2. MATERIALS AND METHODS

2.1. Chemicals and Reagents

Chlorogenic acid, epicatechin, gallic acid, protocatechuic acid, *p*-OH benzoic acid, caffeic acid, vanillic acid, catechin, syringic acid, *p*-coumaric acid, naringenin, rutin, ellagic acid, ferulic acid, apigenin, quercetin, myricetin, luteolin, daidzein, *t*-cinnamic acid, hesperedin, rhamnetin, chrysin, galangin, pinocembrin, and CAPE were obtained from Sigma-Aldrich Chemie (Munich, Germany) and were of HPLC grade. 1,1-diphenyl-2-picrylhydrazyl (DPPH), ferric chloride, Iron (III) chloride hexahydrate, sodium carbonate, FRAP (ferric tripyridyltriazine) reagent, aluminum nitrate nonahydrate, hydrochloric acid, ferrous sulfate, acetic acid, methanol, ethanol, and ammonium acetate were supplied from Merck (Darmstadt, Germany). The Folin-Ciocalteu's reagent was provided by Sigma-Aldrich Chemie (Munich, Germany).

2.2. Sample extraction

Hawthorn samples were acquired from street markets of Sivas/Türkiye in October 2023 (Figure 1). After purchasing, they were instantly placed in the refrigerator until extraction. 50 g of the hawthorn samples were washed with tap water and then thoroughly chopped. 250 mL of 98% methanol was added to chopped hawthorns, and the mixture was agitated for 24 h. After the extract went through filtering with Whatman No. 1 paper, it was kept at –18°C until analysis.

Figure 1. Hawthorn samples from street markets of Sivas/Türkiye in October 2023

2.3. Analysis of Total Phenolic Content (TPC)

The Folin-Ciocalteu method was utilized to evaluate the TPC of hawthorn. (Kemal et al., 2023). After combining 20 µL of methanolic hawthorn extract with 400 µL of 0.2 N Folin-Ciocalteu's reagent, the mixture was diluted with distilled water to 680 µL. Subsequently, after adding Na_2CO_3 (400 mL), the mixture was incubated for two hours at room temperature. Following a 4-minute incubation time, the absorbance of the sample was evaluated at 760 nm using a UV-VIS spectrophotometer. The quantification of hawthorn extract was done using a gallic acid standard curve with a range of 1.000 to 0.0625 mg GAE/mL (y = 1,3228x + 0,0388). The extract's TPC was expressed as mg gallic acid equivalents (GAE) per gram of fresh weight (FW).

2.4. Analysis of Total Flavonoid Content (TFC)

The TFC of hawthorn extract was estimated using the procedure published previously (Fukumoto and Mazza, 2000). In short, 50 µL of 1.0 M NH_4. CH_3COO and 50 µL of 10% Al $(NO_3)_3$ were added to 25 µL of the hawthorn extract. The mixture was subsequently diluted to 3.0 mL using 99% ethanol and incubated for 45 minutes at 25 °C. The absorbance was then measured at 415 nm. TFC was expressed as mg quercetin equivalent (QUE)/100 g FW sample using quercetin standards ranging from 0.050 to 0.0156 mg QUE/mL (y = 4,2446x + 0,0121).

2.5. Determination of Antioxidant Activity

The antioxidant capacity of hawthorn extract was assessed using the ferric-reducing antioxidant power assay (FRAP) and the 2,2-diphenyl-1-picryhydrazyl (DPPH) technique. A fresh FRAP reagent was prepared by combining 25 mL of acetate buffer (300 µM pH:3.6), 2.5 mL of 20 mM FeCl3.6H2O, and 2.5 mL of 10 mM TPTZ (2,4,6-Tris(2-pyridyl)-s-triazine) in 40 mM

HCl. 100 µL of hawthorn extract and 3 mL of FRAP reagent were combined, and the mixture was then incubated at 37 °C for 4 minutes. The absorbance was then recorded at 593 nm. Different concentrations ranging from 1.000-0.0625 µmol/mL Trolox equivalent (TE) were used to plot the standard curve. The results were expressed as µmol TE/g FW sample based on the curve with the corresponding line equation (y=1,5069x+0,0913).

The DPPH radical scavenging activity of hawthorn extract was measured using the method described by Molyneux (Molyneux, 2004). 750 µL of DPPH solution and 750 µL of hawthorn extract were mixed. After 45 min of dark storage at 25 °C, the mixture's absorbance at 517 nm was measured. The sample concentration that led to a 50% reduction in DPPH radical concentration was designated as SC_{50}.

2.6. Determination of Phenolic Profile with HPLC-PDA

The detection of phenolic components in hawthorn extract was performed using reverse-phase high-pressure liquid chromatography (RP-HPLC) (Shimadzu Company LC 20AT, Japan) and a photodiode array (PDA) detector. The RP-HPLC-PDA method utilized in this investigation has already been verified (Kara et al., 2022). C18 column (250 mm x 4.6 mm, 5 µm; GL Sciences) was utilized to separate phenolic components. Acetic acid (2% in water) and acetonitrile (70:30) were used as the mobile phases A and B, respectively. The separation was conducted at 1.0 mL/min flow rate and a column temperature of 30°C. The findings were presented as µg/g of FW (Figure 2).

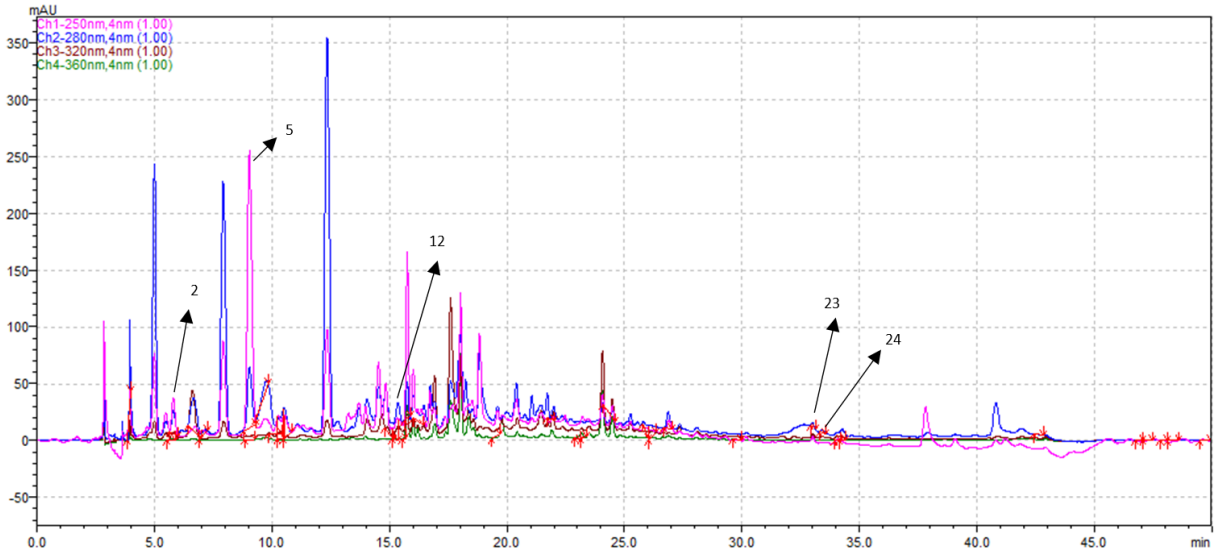

Figure 2. Chromatogram of phenolic components

1. Gallic acid, 2. Protocatechuic acid, 3. Chlorogenic acid, 4. Catechin hydrate, 5. p-OH Benzoic acid, 6. Epicatechin, 7. Caffeic acid, 8. Vanillic acid, 9. Syringic acid, 10. p-Coumaric acid, 11. Rutin, 12. Ellagic acid, 13. Ferulic acid, 14. Myricetin, 15. Daidzein, 16. Luteolin, 17. Quercetin, 18. t-Cinnamic acid, 19. Naringenin, 20. Apigenin, 21. Hesperetin, 22. Rhamnetin, 23. Chrysin, 24. Pinocembrin, 25. CAPE, 26. Galangin

3. RESULTS AND DISCUSSIONS

3.1. Total phenolic and flavonoid content of hawthorn

Oxidative stress preventive and antioxidative effects of hawthorn are attributed to a wide range of phenolic compounds (Turnalar Ülger *et al.*, 2023). Thus, determining the variability of the phenolic content of hawthorn fruit is crucial to obtaining reliable and required quality extracts in terms of chemical content (Çınar *et al.*, 2020). The total phenolic and flavonoid content of hawthorn fruits are presented in Table 1. The hawthorn fruit from Sivas province had a total phenolic content (TPC) of 2.160 mg GAE on a fresh weight (FW) basis. Our finding was significantly higher than those of the natural hawthorn population (0.218-0.711 mg GAE/g FW) in the Suşehri district of Sivas reported by Ağlar et al. (Ağlar *et al.*, 2020). In addition, our finding is close to the lower end of the range of hawthorn genotypes collected from Kayseri province (2.79-3.1 mg GAE/g FW) (Yildiz et al., 2023) and those from the West Mediterranean region of Türkiye (2.83-14.52 mg GAE/g FW) (Çınar et al., 2020). On the other hand, it is significantly lower than the range of 6.6-34.6 mg GAE/g FW collected from Malatya province (Ercişli et al., 2015) and those analyzed from Karaman and Malatya provinces (7.73-8.71 mg GAE/g DW) of Türkiye (Akbulut, 2024) suggesting that phenolic content of hawthorn fruit can vary significantly. The difference in TPC of hawthorn fruits may be attributed to a variety of factors including genotype, species, agronomic techniques, harvest maturity level, climate, postharvest storage, and geographic location. Flavonoids possessing antioxidant activity are one of the primary active ingredients of Crataegus species (Alirezalu *et al.*, 2020). The total flavonoid content (TFC) of hawthorn was found to be 0.193±0.009 mg QE/ g FW (Table 1). Our finding was significantly higher than the TFC of 20 genotypes of hawthorns naturally grown in the flora of Suşehri, a district of Sivas province, in 2019 reported to be in the range of 0.021-0.065 mg QE/ g FW (Ağlar *et al.*, 2020) and that (0.01 mg QE/g DW) reported from Şırnak province of Türkiye (Ceylan *et al.*, 2019). In addition, the TFC of our hawthorn fruits was higher than those of genotypes (G5 and G21) collected from Kayseri province. On the other hand, it was considerably lower than those from the West Mediterranean region of Türkiye (0.56-7.04 mg CE/g FW) (Çınar *et al.*, 2020), those from the Kayseri province (0.2055-0.4759 mg QE/g FW), and that of eastern hawthorn fruits (43.04 mg QE/ g) from Bingöl district (Bengü *et al.*, 2023).

3.2. Antioxidant activities of hawthorn fruit

The antioxidant activity of hawthorn fruit was evaluated using the FRAP and DPPH techniques. Our FRAP value was lower than that of genotypes (15.43-47.23 (mmol TE kg^{-1} FW) cultivated in the Suşehri district of Sivas. The DPPH radical scavenging capacity of hawthorn fruit was measured utilizing SC_{50} values. Decreased SC_{50} values demonstrate a more radical scavenging ability (Dinç *et al.*, 2024). The DPPH value of hawthorn fruit was found to be 3.563 mg/mL which was higher than the ranges (1.48-1.752 mg/mL) reported by Serteser et al. (Serteser *et al.*, 2008) and those (18.31-125.5 µg/mL) reported by Özderin et al. (Ozderin, 2024) indicating lower DPPH scavenging activity of our hawthorn fruits.

Table 1. Total phenolic, total flavonoid, and antioxidant potential of the hawthorn fruits.

	Hawthorn fruit
Total phenolic content (TPC) (mg GAE/ g FW)	2.160±0.069
Total flavonoid content (TFC) (mg QE/ g FW)	0.193±0.009
FRAP (µmol TE/g FW)	0.709±0.028
DPPH SC_{50} (mg/mL)	3.563±0.039

3.3. Phenolic profile of hawthorn fruit

Phenolic acids including gallic, ferulic, syringic, *p*-coumaric, caffeic, and chlorogenic are among the more than 150 bioactive compounds in hawthorn that have been identified (Nazhand *et al.*, 2020). High phenolic composition and widely known antioxidant components consisting of epicatechin, rutin, hyperoside, quercetin, isoquercetin, and protocatechuic acids make *Crataegus* species a good source of antioxidants (Alirezalu *et al.*, 2020). Table 2 summarizes the phenolic compounds detected in hawthorn fruit. Among the 26 phenolic compounds screened, only five phenolic constituents were detected including ellagic acid, protocatechuic acid, *p*-OH benzoic acid, chrysin, and pinocembrin. *p*-OH benzoic acid was the most abundant phenolic compound followed by ellagic acid in hawthorn fruit.

The quantity of *p*-OH benzoic acid, possessing antibacterial, antifungal, antimutagenic, antialgal, antisickling, and estrogenic activity, was identified at the highest concentration in our study (Manuja *et al.*, 2013). Although there is not much data regarding the amount of *p*-OH benzoic acid in hawthorn fruits, the amount of *p*-OH benzoic acid in our study was significantly

higher than those grown in Serbia (0.35-2.07 mg kg^{-1}) analyzed by Natić *et al.*, 2019. Tang et al. (2006) reported that the quantity of 0.48 mg/g in hawthorn is higher than our findings (Tang *et al.*, 2006).

Table 2. Phenolic composition of hawthorn fruits (µg/g FW)

	µg phenolic component /g FW
Gallic acid	-
Protocatechuic acid	1.834
Chlorogenic acid	-
Catechin hydrate	-
p-OH benzoic acid	8.599
Epicatechin	-
Caffeic acid	-
Vanillic acid	-
Syringic acid	-
p-Coumaric acid	-
Rutin	-
Ellagic acid	2.294
Ferulic acid	-
Myricetin	-
Daidzein	-
Luteolin	-
Quercetin	-
t-Cinnamic acid	-
Naringenin	-
Apigenin	-
Hesperidin	-
Rhamnetin	-
Chrysin	0.166
Pinocembrin	0.176
CAPE	-
Galangin	-

* -: not detected

Protocatechuic acid, one of the main metabolites of complex polyphenols (Song *et al.*, 2020), was found to be 1.834 µg/g FW in our study. However, it was not detected in the pulp of *C. monogyna* reported by Mraihi et al. and those grown in two different locations in Serbia (Natić *et al.*, 2019). Natić *et al.*, (2019) reported the protocatechuic acid level of hawthorns in the Radmilovac location of Serbia in the range of 0.78-1.95 mg kg^{-1}, which aligns with our finding. On the other hand, Mraihi et al. (2015) and Zhang et al. (2001) reported the level of protocatechuic acid as 8.61 mg 100 g^{-1} in the pulp of *C. azarolus* (Mraihi *et al.*, 2015) and 3.2 mg/100 g DW in hawthorn fruit, respectively (Zhang *et al.*, 2001) which are higher than our findings. Similarly, the protocatechuic acid level in our study was significantly lower than those (1.7-6.8 mg/100 g FW) of the hawthorn population of the Bahçesaray region in eastern Türkiye reported by Muradoglu et al. (Muradoğlu *et al.*, 2019) and that of reported (3.28 mg/100 g) by Özcan et al. (Özcan *et al.*, 2023).

Ellagic acid, a naturally occurring secondary metabolite of bioactive polyphenolic compounds, was the second phenolic with the highest amount in our hawthorn fruit (Sharifi-Rad *et al.*, 2022). Our finding was higher than that of hawthorns (0.31-0.63 mg kg^{-1}) analyzed by Natić *et al.* (2019) in two different regions of Serbia but lower than that of other regions (2.49-5.58 mg kg^{-1})

Chrysin, a naturally occurring flavonoid that is one of the active ingredients of hawthorn, was discovered to be effective in preventing atherosclerosis (Li *et al.*, 2022). In addition, it has been extensively documented for its potential utility in treating cancer, inflammation, anxiety, and behavioral effects (Ali *et al.*, 2017). Our investigation indicated that hawthorn contains a 0.11 µg/g chrysin. However, to our knowledge, there is no data on the amount of chrysin in hawthorn fruits to compare with published data.

Pinocembrin, one of the promising flavonoids, is widely distributed in many plants, fruits, herbs, fungi, and bee products and has the potential for preventing and treating several diseases such as inflammatory disorders, cancer, ischemic stroke, and cardiovascular diseases (Elbatreek *et al.*, 2023). In our study, the level of pinocembrin in hawthorn was 0.176 µg/g, and as far as we know this is the first pinocembrin amount reported in hawthorn. However, Katanić Stanković et al., analyzed pinocembrin in hawthorn but they didn't detect it (Katanić Stanković *et al.*, 2022).

The results of *p*-coumaric acid, vanillic acid, ferulic acid, syringic acid, gallic acid, catechin, apigenin, and luteolin, which were not detected in the hawthorns we analyzed, are similar to those of Natić *et al.* (2019).

Overall, our findings revealed significant differences in phenolic compounds and antioxidant potential among hawthorn species of previous reports which may be attributed to habitat conditions, genotype, harvesting time, growing temperatures, altitude, light, nutritional components of soil, analytical methods used, and postharvest processing (Alirezalu *et al.*, 2020; Yang and Liu, 2012).

4. CONCLUSION

Our study demonstrated that hawthorn fruits collected from the Sivas province of Türkiye had different phenolic components such as pinocembrin and chrysin than those of previous reports. As far as we know, this is the first study reporting the presence of phenolic components pinocembrin and chrysin in hawthorn fruits. The hawthorn fruits exhibited quantifiable levels of total flavonoid, total phenolic, and antioxidant activity contributing to the expanding body of data about the phytochemical attributes of hawthorn fruits grown in Türkiye. In summary, the assessment of hawthorn fruits may supply essential information for identifying potential cultivars with high bioactive compounds to be used to produce standard extracts for the industry.

DECLARATIONS

The authors declare no conflict of interest**.**

AUTHORS' CONTRIBUTIONS

Yakup KARA was responsible for conducting the experiments and reporting the results. Meryem KARA and Saliha DİNÇ contributed to the preparation of the research framework and writing of the manuscript.

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