



## Phytochemical and Bioactive Assessment of *Crataegus tanacetifolia* and *Crataegus orientalis* Fruit Extracts as a Potential Multi-Enzyme Inhibitor

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### Abstract

Plants are an endless source of bioactive compounds and have been at the centre of research investigating pivotal candidates for plant-derived drugs and dietary supplements. This study evaluated phenolic profiles, triterpenoid contents, enzyme inhibitory activities, antioxidant capacities, and total phenolic content (TPC) of the polar extracts from *Crataegus tanacetifolia* and *Crataegus orientalis*. LCMS/MS and GC-MS analyses revealed 25 phenolic compounds and two triterpenoids in *C. tanacetifolia*, and 26 phenolic compounds and one triterpenoid in *C. orientalis*, with quinic acid as the dominant phenolic compound in both extracts. Oleanolic acid and ursolic acid were detected in the *C. tanacetifolia* extract, whereas only ursolic acid was identified in the *C. orientalis* extract. Both extracts showed strong angiotensin-converting enzyme inhibition activity (~99%), indicating antihypertensive potential, as well as notable urease inhibition activity (*C. tanacetifolia*: 87.08%; *C. orientalis*: 91.66%). The *C. tanacetifolia* and *C. orientalis* extracts also exhibited significant antioxidant activity with an IC50 value of 123.38 and 97.29 µg/mL for DPPH radical scavenging activity and 82.37 and 92.34% for linoleic acid/β-carotene bleaching capacity. The TPC of the extracts was determined to be 26.13 and 76.80 mg GAE/g, respectively. The results showed that *C. tanacetifolia* and *C. orientalis* could be valuable sources of bioactive compounds for pharmaceutical and nutraceutical products.

**Keywords:** *Crataegus tanacetifolia*, *Crataegus orientalis*, Phenolic Profile, Triterpenoid Content, Enzyme Inhibition, Antioxidant Capacity



## Potansiyel Çoklu Enzim İnhibitörü Olarak *Crataegus tanacetifolia* ve *Crataegus orientalis* Meyve Ekstraktlarının Fitokimyasal ve Biyoaktif Değerlendirmesi

### Öz

Bitkiler, biyoaktif bileşiklerin sonsuz kaynağı olup bitki kökenli ilaçlar ve besin takviyeleri geliştirmeye yönelik araştırmaların odağında yer almaktadır. Bu çalışmada, *Crataegus tanacetifolia* ve *Crataegus orientalis* 'ten elde edilen polar ekstraktların fenolik profilleri, triterpenoid içeriği, enzim inhibisyon aktiviteleri, antioksidan kapasiteleri ve toplam fenolik içerikleri (TPC) değerlendirilmiştir. LCMS/MS ve GC-MS analizleri sonucunda, *C. tanacetifolia* 'da 25 adet fenolik bileşik ve iki adet triterpenoid, *C. orientalis* 'te ise 26 adet fenolik bileşik ve bir adet triterpenoid tespit edilmiştir. Her iki ekstrakta da baskın fenolik bileşiğin kinik asit olduğu belirlenmiştir. *C. tanacetifolia* ekstraktında oleanolik asit ve ursolik asit saptanırken, *C. orientalis* ekstraktında yalnızca ursolik asit tespit edilmiştir. Her iki ekstrakt da güçlü anjiyotensin dönüştürücü enzim inhibisyon aktivitesi (~%99) göstererek

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antihipertansif potansiyeli ve ayrıca dikkate değer üreaz inhibisyon aktivitesi (*C. tanacetifolia*: %87,08; *C. orientalis*: %91,66) göstermiştir. *C. tanacetifolia* ve *C. orientalis* ekstraktları, DPPH radikal süpürme aktivitesi için sırasıyla 123,38 ve 97,29 µg/mL IC<sub>50</sub> değerleri ile linoleik asit/β-karoten ağartma testinde %82,37 ve %92,34 oranlarında önemli antioksidan aktivite göstermiştir. Ekstraktların TPC değerleri sırasıyla 26,13 ve 76,80 mg GAE/g olarak belirlenmiştir. Sonuçlar, *C. tanacetifolia* ve *C. orientalis*'in farmasötik ve nutrasötik ürünlerin geliştirilmesinde kullanılabilecek değerli biyoaktif bileşik kaynakları olabileceğini ortaya koymuştur.

**Anahtar kelimeler:** *Crataegus tanacetifolia* , *Crataegus orientalis* , Fenolik Profil, Triterpenoid İçerik, Enzim İnhibisyonu, Antioksidan Kapasite



## 1. Introduction

Enzyme inhibitors represent a critical advancement in modern medicine and constitute a powerful pharmacological approach for developing therapeutics targeting enzymes responsible for disease progression [1]. Although enzyme inhibitors can be obtained through the synthesis of new chemical compounds or by modifying the structures of existing molecular scaffolds, natural sources remain among the most important sources of new enzyme inhibitors [2]. A large number of enzyme inhibitors exist in nature, waiting to be discovered, offering a unique structural diversity that could improve treatment quality and overall quality of life.

Natural products have been employed in traditional medicine for centuries to treat many diseases and ailments. Over time, classical chemical approaches to natural products have led to the discovery of a wide variety of bioactive secondary metabolites, many of which have become important drug candidates. Over the past few years, more than 60% of new anticancer drugs and 75% of drugs used to treat infectious diseases have come from natural sources [3]. Furthermore, more than 25% of all prescription drugs originate from plants [4]. Despite the vast diversity of plant species available, it is estimated that only a small fraction of existing natural resources has been scientifically evaluated, highlighting the enormous potential of this rich and largely unexplored resource [5].

Phytochemicals, also referred to as bioactive compounds, are naturally occurring substances found in different parts of plants and have gained considerable attention due to their wide range of beneficial properties. Polyphenols, the most diverse group of phytochemicals, provide numerous pharmacological properties in the prevention of various diseases, including antioxidant, anticancer, anti-inflammatory, antihypertensive, and antimicrobial properties [6]. Beyond these well-known effects, polyphenols can also regulate the activity of many enzymes and cellular receptors, although the mechanisms underlying these interactions are not yet fully understood. These biological activities are attributed to the diverse phytochemical content present in plants [2,7].

*Crataegus* species belong to the Rosaceae family and these deciduous, spring-flowering shrubs are native to North America, East Asia, Central Asia, and Europe. Extracts derived from *Crataegus* species have long been recognized for their cardiogenic, sedative, and astringent properties. Furthermore, hawthorn-based products are widely promoted as alternative therapeutic options for conditions such as hypertension, angina, arrhythmia, and early-stage congestive heart failure [8]. Owing to their high flavonoid and triterpenic acid content, these species have gained increasing commercial importance and are now commonly incorporated into dietary supplements and pharmaceutical formulations across many countries [9,10].

Turkey is home to 21 different *Crataegus* species, many of which remain poorly explored from a scientific perspective, particularly regarding their enzyme-inhibitory properties [11,12]. Despite the widespread traditional use of *Crataegus* species, comprehensive phytochemical and bioactivity-oriented studies on several endemic or less-studied taxa are still limited. Therefore, the present study was designed to investigate the biological potential of *C. tanacetifolia* and *C. orientalis*, two species for which detailed enzyme inhibition data remain scarce.

This study provides a comprehensive phytochemical profiling of the fruit extracts of these species using 53 phenolic and seven triterpenoid reference standards. The enzyme inhibitory activities

of the extracts were systematically assessed against seven clinically relevant enzymes (angiotensin-converting enzyme, urease, elastase, collagenase, acetylcholinesterase, butyrylcholinesterase, and tyrosinase), which are closely associated with neurodegenerative and related disorders. Furthermore, the antioxidant activity and total phenolic content (TPC) of the extracts were evaluated. The findings of this study contribute new insights into the phytochemical composition and multi-target bioactivity of these *Crataegus* species, supporting their potential value as natural sources of bioactive compounds.

## 2. Material and Method

### 2.1. Material

Fresh wild fruits of *C. tanacetifolia* and *C. orientalis* were obtained from a local marketplace in Nevşehir, Türkiye. The samples were visually inspected, cleaned with sterile distilled water, and lyophilized until the moisture level was reduced to below 14%, in accordance with established protocols [13]. The resulting dried materials were sealed and stored at  $-80\text{ }^{\circ}\text{C}$  in the dark until use.

Moisture content was quantified gravimetrically, and the results were reported as percentage (%) using Equation (1).

$$\text{Moisture (\%)} = (\text{fresh weight} - \text{dry weight}) / \text{fresh weight} \times 100 \quad (1)$$

### 2.2. Preparation of polar extracts of *C. tanacetifolia* and *C. orientalis*

A total of 25 g of dried fruit material was first ground into a fine powder using a laboratory blender (Waring Commercial Blender, USA). From the resulting homogenized powder, a 3 g portion was weighed and mixed with 20 mL of deionized water. The mixture was subjected to ultrasound-assisted extraction in a water bath (SK06GT Kudos Ultrasonic Water Bath, Korea) for 30 min at 30–40  $^{\circ}\text{C}$ . The extract was then centrifuged at  $8,000\times g$  for 15 min (Hanil Science Industrial Combi 514R, Korea) to obtain the supernatant. This extraction step was repeated three times, and the collected supernatants were pooled and stored at  $-80\text{ }^{\circ}\text{C}$  until further analysis [14].

### 2.3. Determination of polyphenolic profile of polar extracts of *C. tanacetifolia* and *C. orientalis*

The phenolic profile of the extracts was examined using 53 phenolic compound standards on a Shimadzu Nexera UHPLC (ultra-high performance liquid chromatography) system, coupled with a Shimadzu LCMS-8040 triple quadrupole mass spectrometer, according to the method developed and validated by Yilmaz et al. (2018, 2020) [15,16].

### 2.4. Determination of the triterpenoid contents of polar extracts of *C. tanacetifolia* and *C. orientalis*

The triterpenoid composition of the extracts was characterized using gas chromatography–mass spectrometry (GC–MS) with seven triterpenoid standards. The analyses were carried out on an Agilent 7890 gas chromatograph equipped with an Agilent 5977B mass spectrometer. Separation was achieved using an HP-5MS capillary column (30 m  $\times$  0.25 mm  $\times$  0.25  $\mu\text{m}$  film thickness), with helium employed as the carrier gas at a constant flow rate of 1 mL/min and a pressure of 20 psi. A 1.0  $\mu\text{L}$  aliquot of each sample was injected with a split ratio of 1:10. Electron ionization was carried out at 70 eV, and mass spectra were recorded in full-scan mode over an  $m/z$  range of 50–800. Prior to analysis, the samples were derivatized with N,O-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane. The final concentration of the derivatized samples was adjusted to 1000 mg/L [17].

### 2.5. Bioactivity assays of polar extracts of *C. tanacetifolia* and *C. orientalis*

#### 2.5.1. Determination of enzyme inhibitory activities

The inhibition activities of angiotensin-converting enzyme (ACE), urease, elastase, collagenase, tyrosinase, acetylcholinesterase and butyrylcholinestase in the extracts were determined according to the protocol reported by Findik et al. (2024) [18].

### 2.5.2. The antioxidant capacity of polar extracts of *C. tanacetifolia* and *C. orientalis*

The antioxidant capacity of fruit extracts was examined using DPPH free radical scavenging and the linoleic acid/ $\beta$ -carotene bleaching assay [19]. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as standards.

### 2.5.3. Total Phenolic Content (TPC) of polar extracts of *C. tanacetifolia* and *C. orientalis*

The TPC of the fruit extracts was measured using the Folin-Ciocalteu method [19]. Gallic acid was used as the standard. The results were given as mg GAE/g.

### 2.6. Statistical analysis

The results are presented as the mean value from three replications, with standard errors (S.E.). A one-way analysis of variance (ANOVA) was conducted to assess the statistical significance of the differences between the extracts. The Duncan multiple-comparison test was used to compare mean values; differences were considered significant at  $p < 0.05$ . The statistical analysis was performed using SPSS Statistics software, version 22.00 (SPSS Inc., Chicago, IL, USA).

## 3. Results

### 3.1. Phenolic profiles of the polar extracts of *C. tanacetifolia* and *C. orientalis*

The phenolic contents of the polar extracts of *C. tanacetifolia* and *C. orientalis* were evaluated by scanning the presence of 53 standard phenolic compounds. Among the identified phenolic compounds, 25 phenolics were detected in the polar extract of *C. tanacetifolia*, whereas 26 phenolics were identified in the polar extract of *C. orientalis* (Table 1). Twenty-two compounds were common to both species. Gentisic acid, salicylic acid, and genistin were detected exclusively in the polar extract of *C. tanacetifolia*, while o-coumaric acid, isoquercitrin, daidzin, and genistein were detected only in the polar extract of *C. orientalis*.

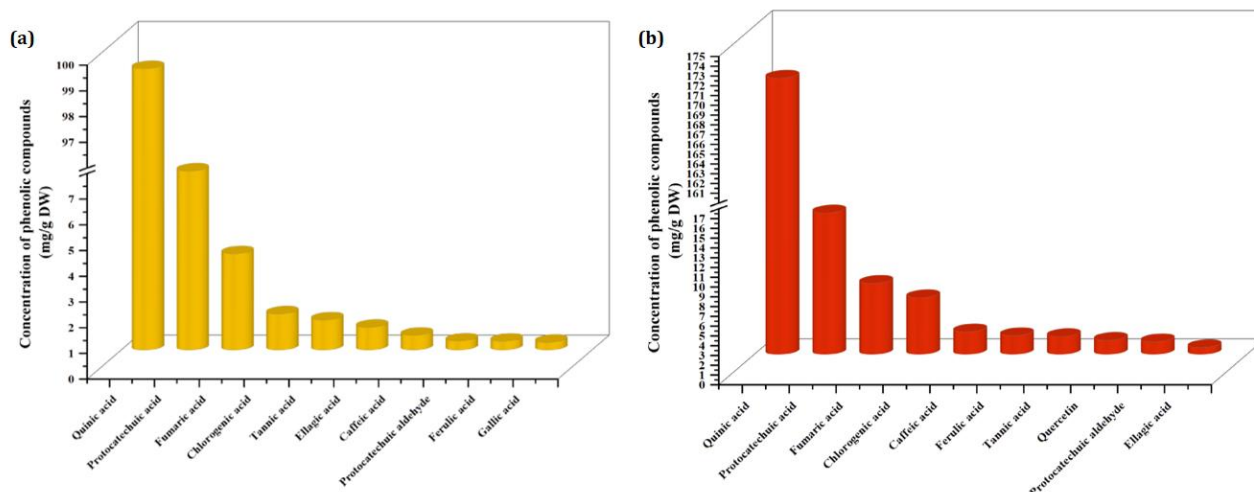
**Table 1.** Phenolic compounds of the polar extracts of *C. tanacetifolia* and *C. orientalis*.

Reference Phenolic Compound	Parent Ion (m/z) <sup>a</sup>	MS <sup>2</sup> (Collision Energy) <sup>b</sup>	Quantification (mg compound/g extract)	
			<i>C. tanacetifolia</i>	<i>C. orientalis</i>
<b>Simple Phenols</b>				
<b>Phenolic acids</b>				
<b>Hydroxycinnamic acids</b>				
Caffeic acid	179.0	134.0	0.577 ± 0.009	2.305 ± 0.350
Chlorogenic acid	353.0	85.0	1.410 ± 0.030	5.798 ± 0.123
o-Coumaric acid			ND	0.063 ± 0.003
p-Coumaric acid	163.0	93.0	0.117 ± 0.002	0.108 ± 0.002
Ferulic acid	192.8	149.0	0.347 ± 0.006	1.917 ± 0.035
Quinic acid	190.8	93.0	98.740±3.673	169.669±6.312
<b>Hydroxybenzoic acids</b>				
Ellagic acid	301.0	284.0	0.889 ± 0.032	0.700 ± 0.026
Gallic acid	168.8	79.0	0.303 ± 0.034	0.547 ± 0.006
Gentisic acid	152.8	109.0	0.168 ± 0.003	ND
Protocatechuic acid	152.8	108.0	6.967 ± 0.244	14.473 ± 0.507
Salicylic acid	137.2	65.0	0.012 ± 0.000	ND
<b>Coumarins</b>				
Coumarin	146.9	103.1	0.078 ± 0.003	0.080 ± 0.003
<b>Polyphenols</b>				
<b>Flavonoids</b>				
<b>Flavones</b>				
Apigenin	268.8	151.0/149.0	0.007 ± 0.000	0.003 ± 0.000
Luteolin	284.8	151.0/175.0	0.088 ± 0.003	0.032 ± 0.001
Cynaroside	447.0	284.0	0.026 ± 0.001	0.027 ± 0.001
<b>Flavonols</b>				
Kaempferol	285.0	239.0	0.064 ± 0.001	0.597 ± 0.013
Isoquercitrin			ND	0.529 ± 0.012

Quercetin	301.0	272.9	0.152 ± 0.004	1.449 ± 0.039
<b>Isoflavonols</b>				
Daidzin			ND	0.063 ± 0.001
Genistein			ND	0.003 ± 0.000
Genistin	431.0	239.0	0.013 ± 0.000	ND
<b>Flavanones</b>				
Hesperetin	301.0	136.0/286.0	0.011 ± 0.000	0.112 ± 0.004
Naringenin	270.9	119.0	0.005 ± 0.000	0.025 ± 0.000
<b>Non-Flavonoids</b>				
<b>Tannins</b>				
Tannic acid	182.8	78.0	1.175 ± 0.022	1.823 ± 0.035
<b>Hydroxybenzaldehydes</b>				
Protocatechuic aldehyde	137.2	92.0	0.354 ± 0.014	1.289 ± 0.051
Syringaldehyde	181.0	151.1	0.056 ± 0.001	0.063 ± 0.001
Vanillin	153.1	125.0	0.175 ± 0.002	0.227 ± 0.003
<b>Organic acids</b>				
Fumaric acid	115.2	40.9	3.749 ± 0.034	7.267 ± 0.070
Aconitic acid	172.8	129.0	0.075 ± 0.002	0.033 ± 0.001
Rutin-D3-IS <sup>d</sup>	612.2	304.1	IS	
Ferulic acid-D3-IS <sup>d</sup>	196.2	152.1	IS	
Quercetin-D3-IS <sup>d</sup>	304.0	275.9	IS	

ND: Not determined. IS: Internal standard. <sup>a</sup> MI (m/z): Molecular ions of the standard analytes (m/z ratio). <sup>b</sup> FI (m/z): Fragment ions. Acacetin, amentoflavone, astragalin, catechin, chrysin, cosmosiin, daidzein, epicatechin, epicatechingallate, epigallocatechin, epigallocatechingallate, fisetin, hesperidin, miquelianin, nicotiflorin, piceid, quercitrin, rosmarinic acid, rutin, sinapic acid, syringic acid, vanilic acid, 1,5-dicaffeoylquinic acid, and 4-oh benzoic acid were not detected in the extracts.

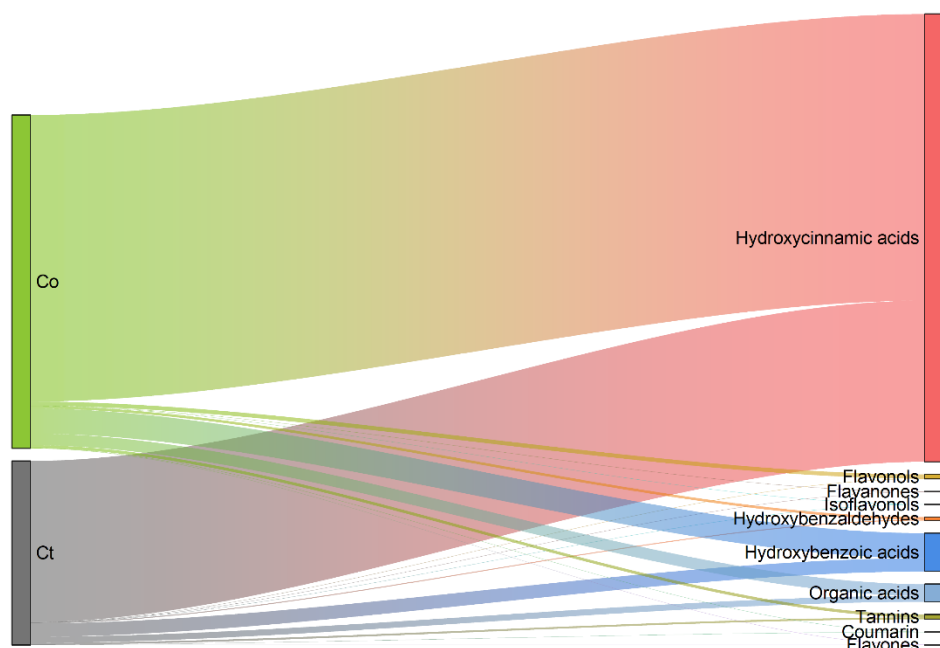
The phenolic acids present at the highest levels in both extracts, in decreasing order of concentration, were quinic acid, protocatechuic acid, fumaric acid, and chlorogenic acid (Figure 1). For each of these compounds, higher concentrations were detected in the *C. orientalis* extract. These were followed by tannic acid, ellagic acid, and caffeic acid in *C. tanacetifolia*, whereas caffeic acid, ferulic acid, and tannic acid were among the predominant phenolic acids in *C. orientalis*.



**Figure 1:** Ranking of the ten most abundant phenolic compounds in the polar extracts of *C. tanacetifolia* (a) and *C. orientalis* (b).

The Sankey diagram visualization (Figure 2) clearly illustrated the distribution of phenolic subgroups in the polar extracts. Hydroxycinnamic acids were identified as the dominant phenolic subgroup in both species; however, they were more abundant in the *C. orientalis* extract. Hydroxybenzoic acids constituted the second most abundant phenolic subgroup in both species, following hydroxycinnamic acids.

With respect to flavonoids, flavonols were predominant in the *C. orientalis* extract, whereas they were detected at very low levels in the *C. tanacetifolia* extract. In addition, tannins, hydroxybenzaldehydes, and organic acids were observed to be more prevalent in the *C. orientalis* extract.



**Figure 2:** Sankey diagram showing the relationships and relative contributions of phenolic subclasses in the polar extracts of *C. tanacetifolia* and *C. orientalis*.

### 3.2. Triterpenoid contents of the polar extracts of *C. tanacetifolia* and *C. orientalis*

The extracts were screened for the existence of seven triterpenoid compounds, and the results are presented in Table 2. Both oleanolic acid and ursolic acid were observed in the *C. tanacetifolia* extract among the compounds analyzed, whereas only ursolic acid was identified in the *C. orientalis* extract. Quantitative analysis revealed that the polar extract of *C. tanacetifolia* was particularly rich in triterpenoids, containing high levels of oleanolic acid (2810.078  $\mu\text{g/g DW}$ ) and ursolic acid (4722.092  $\mu\text{g/g DW}$ ).

**Table 2.** Triterpenoid contents of the polar extracts of *C. tanacetifolia* and *C. orientalis* by GC-MS

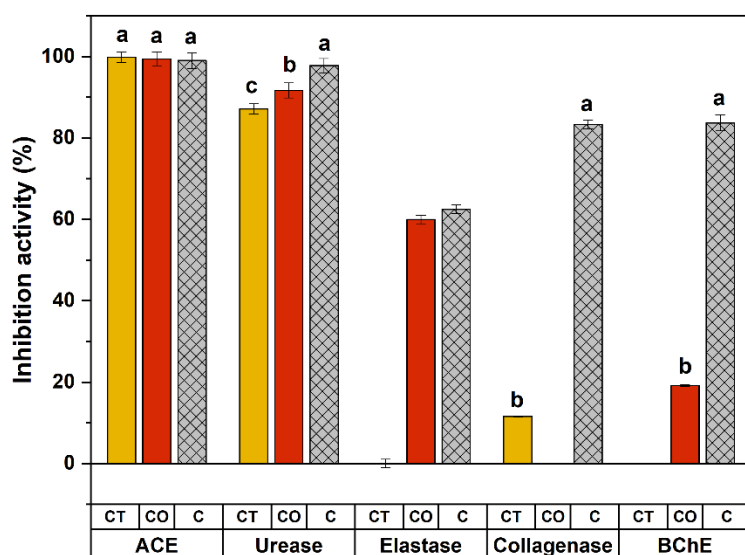
Compounds	R <sub>t</sub> <sup>a</sup>	Molecular ion- m/z (relative intensity %) (m/z) <sup>b</sup>	% RSD <sup>c</sup>	Three major fragment ions m/z (relative intensity %)			<i>C. tanacetifolia</i> ( $\mu\text{g/g DW}$ )	<i>C. orientalis</i> ( $\mu\text{g/g DW}$ )
Alphaamyrin	17.99	498 (2.5)	0.025	218(100)	203(16.6)	189(18.3)	ND	ND
Moronic acid	20.71	527 (21.1)	0.029	189(100)	203(40.3)	409(24.3)	ND	ND
Oleanonic acid	20.96	527 (12.3)	0.023	203(100)	408(64.5)	189(52.6)	ND	ND
Oleanolic acid	21.55	601 (2.3)	0.026	203(100)	189(31.5)	320(28.6)	2810.078	ND
Betulinic acid	21.90	601 (4.9)	0.019	189(100)	203(34.5)	320(21.8)	ND	ND
Ursolic acid	22.55	601 (2.3)	0.015	203(100)	189(32.9)	320(79.6)	4722.092	2085.562
Ursonic acid	22.91	527 (9.5)	0.028	203(100)	320(60.4)	189(24.9)	ND	ND

<sup>a</sup>R<sub>t</sub>: Retention time; <sup>b</sup>Mother ion(m/z): Molecular ions of the standard compounds (m/z ratio); <sup>c</sup>RSD: Relative standard deviation.

ND: Not detected.

### 3.3. The enzyme inhibition activities of the polar extracts of *C. tanacetifolia* and *C. orientalis*

The inhibitory potentials of the polar extracts of *C. tanacetifolia* and *C. orientalis* were evaluated against seven enzymes that are the key targets in the therapeutic management of associated pathologies and the results are presented in Figure 3. Both extracts demonstrated remarkable inhibitory activity against ACE, exhibiting inhibition rates exceeding 99%. This activity was comparable to that of the commercial standard lisinopril, indicating strong ACE-inhibitory potential. The polar extracts of *C. tanacetifolia* and *C. orientalis* inhibited urease activity by 87.08% and 91.66%, respectively ( $p < 0.05$ ), whereas the standard inhibitor thiourea showed an inhibition level of 97.74%. Only the polar extract of *C. orientalis* showed elastase inhibitory activity comparable to that of the standard oleanolic acid (59.93% vs. 62.45%). Similarly, inhibitory activity against BChE was detected only in the *C. orientalis* extract (19.12%). In contrast, the polar extract of *C. tanacetifolia* exhibited moderate collagenase inhibitory activity, with an inhibition value of 11.57%. No inhibitory activity against tyrosinase and AChE was observed for either extract.

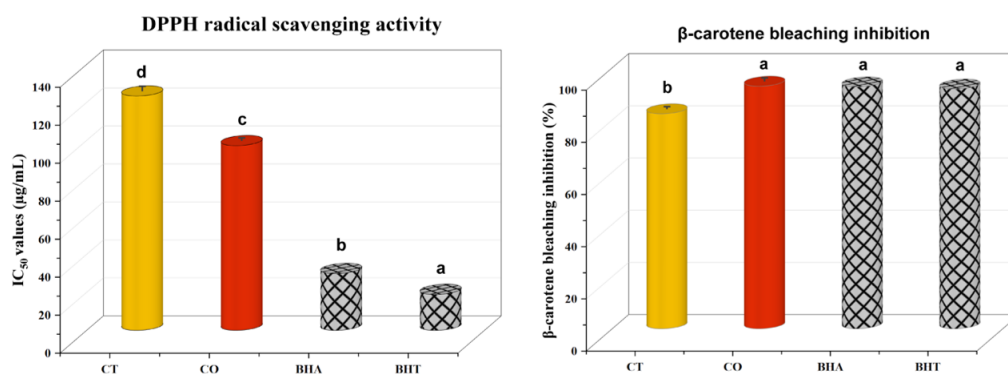


**Figure 3.** Enzyme inhibitory activities of the polar extracts of *C. tanacetifolia* and *C. orientalis*. Values are expressed as mean  $\pm$  standard error. CT: *C. tanacetifolia*; CO: *C. orientalis*; C: control (for ACE: Lisinopril; for urease: Thiourea; for elastase: Oleanolic acid, for collagenase: Epicatechin gallate; for BChE: Galanthamine). Different letters indicate a significant difference between the samples ( $p < 0.05$ ).

### 3.4. Antioxidant activities and TPC of the polar extracts of *C. tanacetifolia* and *C. orientalis*

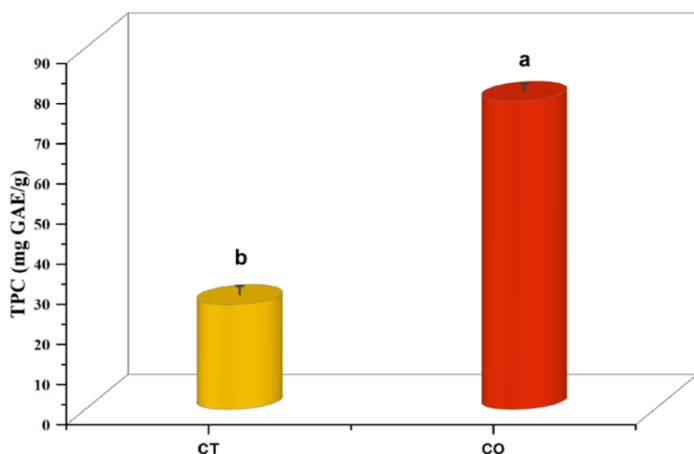
The antioxidant activities of the polar extracts of *C. tanacetifolia* and *C. orientalis* were assessed using the DPPH and  $\beta$ -carotene methods, and the results are presented in Figure 4. The  $IC_{50}$  values of the polar extracts of *C. tanacetifolia* and *C. orientalis*, as determined by the DPPH assay, were 123.38 and 97.29  $\mu$ g DW/mL, respectively.

According to the  $\beta$ -carotene method, *C. tanacetifolia* and *C. orientalis* extracts exhibited strong antioxidant capacity, with inhibition percentages of 82.32% and 92.94%, respectively, approaching the levels of the standards BHA (92.69%) and BHT (91.84%).



**Figure 4:** Antioxidant activity of the polar extracts of *C. tanacetifolia* (yellow bar) and *C. orientalis* (red bar). Values are expressed as mean  $\pm$  standard error. Different letters indicate a significant difference between samples ( $p < 0.05$ ).

The TPC of the extracts is presented in Figure 5. The TPC of the *C. tanacetifolia* extract was found to be  $26.13 \pm 2.14$  mg GAE/L, while that of the *C. orientalis* extract was found to be  $76.80 \pm 1.87$  mg GAE/L.



**Figure 5.** TPC of the polar extracts of *C. tanacetifolia* (yellow bar) and *C. orientalis* (red bar). Values are expressed as mean  $\pm$  standard error. Different letters indicate a significant difference between samples ( $p < 0.05$ ).

#### 4. Discussion and Conclusion

##### 4.1. Phenolic contents of the polar extracts of *C. tanacetifolia* and *C. orientalis*

Phenolic compounds are natural, bioactive plant molecules that exhibit antioxidant, antimicrobial, anti-inflammatory, and antiproliferative activities. These properties have generated significant interest in the use of plants across many industries. Among different plant tissues used in plant-based products, fruits are particularly prominent due to their high phenolic content [20].

The results of the present study demonstrated that the fruits of both *Crataegus* species investigated were rich in phenolic compounds, with diversity and abundance. In particular, both species were characterized by a high level of phenolic acids, with hydroxycinnamic acids being the predominant group. Quinic acid, caffeic acid, chlorogenic acid, *o*-coumaric acid, *p*-coumaric acid, and ferulic acid were the hydroxycinnamic acids that were detected in the extracts. Among these compounds, quinic acid was the dominant species in both extracts.

Quinic acid, a cyclohexanecarboxylic acid, exhibits a wide range of biological activities, including antioxidant, antidiabetic, anticancer, antimicrobial, antiviral, anti-aging, protective, antinociceptive, and analgesic effects, as demonstrated by numerous *in vitro* and *in vivo* pharmacological studies [21]. Given that quinic acid was identified as the predominant phenolic compound in both *Crataegus* extracts, it may contribute substantially to the observed bioactivities. Although there are few reports that specifically quantify quinic acid in the fruits of *C. tanacetifolia* and *C. orientalis*, quinic acid and its derivatives have been identified as prominent phenolic constituents in the fruits of other *Crataegus* species, such as *C. monogyna*, using LC-MS/MS analysis [22]. This suggests that this compound is widespread across the genus.

Along with quinic acid, caffeic acid, and chlorogenic acid were identified as prominent phenolic acids in the extracts. Chlorogenic acid and caffeic acid play important roles in disease prevention. Previous studies have reported that these two compounds exhibit numerous health-promoting effects, including antioxidant, anti-inflammatory, antidiabetic, antiviral, and anticancer activities. They have been shown to reduce oxidative stress, modulate inflammatory responses, and inhibit carcinogenic processes in both *in vitro* and *in vivo* models. Furthermore, chlorogenic acids have demonstrated protective effects against metabolic and cardiovascular disorders, such as diabetic nephropathy and atherosclerosis, and have also been associated with antihypertensive and antiarrhythmic activities [23]. Therefore, *Crataegus* species rich in these compounds may significantly contribute to the therapeutic potential of phenolic-rich plant extracts. Notably, *C. orientalis* exhibited higher levels of both caffeic acid and chlorogenic acid compared to *C. tanacetifolia*.

When the hydroxybenzoic acid profiles of the samples were examined, protocatechuic acid emerged as a prominent compound and was detected at substantially higher levels in *C. orientalis* fruits. Previous studies have reported protocatechuic acid levels in *C. orientalis* fruit extracts ranging from 7 to 11  $\mu\text{g/g DM}$  [24], whereas a markedly higher concentration (14.473 mg/g DW) was detected in the present study. This discrepancy may be attributed to differences in extraction solvents, analytical techniques, or geographical and environmental factors.

When individual flavonoids were evaluated, *C. orientalis* was found to exhibit a richer flavonoid profile, particularly with respect to flavonols, including kaempferol, isoquercitrin, and quercetin. Among these compounds, quercetin was especially prominent in *C. orientalis*, occurring at notably higher levels.

In addition to these compounds, the relatively high contents of tannic acid and fumaric acid further highlight the richness of the phenolic and organic acid profiles of both extracts.

#### 4.2. Triterpenoid contents of the polar extracts of *C. tanacetifolia* and *C. orientalis*

Triterpenoids are a class of terpenes characterized by a carbon skeleton composed of six isoprene units and exhibit significant pharmacological activities, representing a structurally diverse group with over 20,000 known members [25]. Comparative analysis of triterpenoid content between the two species revealed that *C. tanacetifolia* exhibited greater diversity and higher levels of triterpenoids, as determined by the seven reference standards used in the screening. While only ursolic acid was detected in *C. orientalis*, the fruits of *C. tanacetifolia* were identified as a rich source of both ursolic and oleanolic acids. Although comprehensive triterpenoid profiling of *Crataegus tanacetifolia* and *C. orientalis* has not been extensively reported in the literature, studies on other *Crataegus* species have identified a range of ursane-type triterpenoids, supporting the relevance of profiling these compounds in hawthorn fruits [26].

#### 4.3. The enzyme inhibition activities of the polar extracts of *C. tanacetifolia* and *C. orientalis*

Hypertension is a major risk factor contributing to morbidity and mortality in cardiovascular diseases and is associated with severe complications, including stroke, atherosclerosis, myocardial infarction, and renal disorders. ACE, a key component of the renin-angiotensin-aldosterone system, plays an important role in the regulation of hypertension. ACE increases blood pressure by converting

inactive angiotensin I into angiotensin II, a potent vasoconstrictor. The antihypertensive action of ACE inhibitors, including captopril, enalapril, and lisinopril, is primarily mediated by inhibition of angiotensin II synthesis. However, these drugs can cause various side effects, such as hypotension, cough, taste disturbances, and allergic reactions. Therefore, natural ACE inhibitors and natural hypertension regulators, which have fewer side effects, are emerging as a potential and reliable alternative to synthetic drugs [27].

The pronounced ACE inhibitory activity observed in both extracts suggests that *Crataegus* species may represent promising natural sources for the management of hypertension. This bioactivity is likely associated with the high phenolic content of the extracts, as phenolic compounds are well known for their ability to modulate enzyme activity [28]. Similar ACE inhibitory effects have previously been reported for other *Crataegus* species [29,30], supporting the relevance of the present findings.

Urease plays an important role in nitrogen metabolism in various organisms by hydrolysing urea into ammonia. However, excessive urease activity can lead to pathologies such as peptic ulcers, nephropathy, and stomach cancer, as well as agricultural issues such as ammonium depletion in soil and reduced nitrogen use efficiency [31]. Therefore, the inhibition of urease activity has emerged as an important factor in human health complications, as well as in agriculture and biotechnology. This has led to extensive research into potential inhibitors.

In the present study, both extracts demonstrated pronounced inhibitory activity against urease. This observed inhibition activity may be attributed not only to their rich phenolic composition but also to the presence of triterpenoid compounds. Both phenolic acids and flavonoids, as well as triterpenoids such as ursolic and oleanolic acids, have been reported to exhibit urease inhibitory properties [32,33]. The combined presence of these bioactive constituents may result in additive or synergistic effects, thereby enhancing the extracts' anti-ureolytic potential. The ability of both extracts to suppress ureolytic activity highlights their potential as natural sources of urease inhibitors and provides a basis for further studies aimed at isolating and characterizing bioactive anti-ureolytic constituents.

Elastase is a potent serine protease that breaks down elastin, an important component found in the lungs, blood vessel walls, and other organs. However, excessive elastin breakdown by elastase contributes to the development of many common inflammatory diseases, including emphysema, chronic bronchitis, hepatitis, rheumatoid arthritis, and various cardiovascular and cerebrovascular diseases. Consequently, elastase inhibitors are considered an effective anti-inflammatory treatment [34]. The polar extract of *C. orientalis* is notable for its elastase inhibitory activity, which is comparable to that of the commercial standard, oleanolic acid.

#### **4.4. Antioxidant activity and total phenolic content of polar extracts from *C. tanacetifolia* and *C. orientalis***

Antioxidant capacity varies among plant species and is largely influenced by their phytochemical composition. In the present study, *C. orientalis* demonstrated stronger radical scavenging activity than *C. tanacetifolia*, which may be attributed to its higher TPC.

Although comprehensive comparisons of antioxidant activity focusing specifically on *C. tanacetifolia* and *C. orientalis* are scarce, studies involving multiple *Crataegus* species have demonstrated significant inter-species variation in antioxidant capacity, with *C. orientalis* typically exhibiting measurable activity among hawthorn fruits [12].

This study comparatively evaluated the phytochemical composition and bioactive properties of polar fruit extracts from *C. tanacetifolia* and *C. orientalis*. Overall, *C. orientalis* exhibited a richer profile in terms of both phenolic compounds diversity and abundance, as well as stronger enzyme inhibitory activity, antioxidant capacity, and higher total phenolic content. In contrast, *C. tanacetifolia* was distinguished by its higher triterpenoid content.

The pronounced inhibitory activities of both extracts against key enzymes, particularly ACE, urease, and elastase, highlighted the potential of these *Crataegus* fruits as promising multi-target enzyme inhibitors. These findings supported the growing interest in *Crataegus* species as valuable

natural sources of bioactive compounds. Nevertheless, further comprehensive studies are required to identify the specific compounds responsible for the observed bioactivities and to better elucidate the therapeutic potential of hawthorn fruits endemic to Turkey, particularly for pharmaceutical and nutraceutical applications.



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This work is original.

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