



LC-MS/MS Analysis and Biological Activities of Different Parts of *Ziziphora capitata* L.

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ABSTRACT

The *Ziziphora* species, classified under the Lamiaceae family, have a strong aromatic property. *Ziziphora* species have been used in folk medicine as sedative, gastric, aphrodisiac, bloating, and degassing. In the current study, the phenolic and flavanoid content of ethanol extracts of *Ziziphora capitata* L. species of flower, leaf, branch, mixed, and root parts was determined by the LC-MS/MS device. In addition, the antioxidant and cytotoxic activities of the extracts, as well as their inhibitory effects on enzymes (antihypertensive, AchE (acetylcholinesterase), BchE (butyrylcholinesterase), elastase, tyrosinase, collagenase and urease), were determined. The LC-MS/MS results showed that quinic acid (25578, 5842, 25171, 14055, 10597 $\mu\text{g g}^{-1}$, respectively) was found in higher amounts in flower, leaf, branch, mixed, and root extracts of *Z. capitata* species compared to other components. Additionally, rosmarinic acid (17097 $\mu\text{g g}^{-1}$), cynaroside (8432), and hesperidin (8067) were found to be major components. It was observed that the flower extract of the species exhibited strong antioxidant activity (IC_{50} : 37.18 \pm 1.36 $\mu\text{g mL}^{-1}$, 9.89 \pm 0.45, $\text{A}_{0.5}$: 16.27 \pm 0.02, respectively) in DPPH, ABTS and CUPRAC methods. It was concluded that the leaf extract of *Z. capitata* species had a strong cytotoxic effect on HT-29 (colon cancer cell line) (viability %: 9.26 \pm 0.69). It was observed that the root part of the species exhibited higher activity in butyrylcholinesterase (BChE) enzyme inhibition activity (inhibition %: 40.56 \pm 0.88) than other parts. It was determined that *Z. capitata* extracts did not show acetylcholinesterase, urease, tyrosinase, elastase, collagenase, and antihypertensive enzyme activity or showed low activity. As a result, it is thought that the flower extract of the *Z. capitata* species has better results in terms of the examined parameters, whereas the leaf extract needs to be subjected to more detailed *in vitro* and *in vivo* research conducted to be used in the pharmaceutical industry as a result of its cytotoxic effect against colon cancer cell lines.

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Ziziphora capitata L. Türünün Farklı Kısımlarının LC-MS/MS Analizi ve Biyolojik Aktiviteleri

ÖZET

Lamiaceae familyasının altında sınıflandırılan *Ziziphora* türleri güçlü aromatik özelliğe sahiptir. Halk hekimliğinde *Ziziphora* türleri yatıştırıcı, midevi, afrodisyak, şişkinlik ve gaz giderici olarak kullanılmıştır. Mevcut çalışmada *Ziziphora capitata* L. türünün çiçek, yaprak, dal, karışık ve kök kısımlarının etanol ekstratlarının fenolik ve flavanoid içeriği LC-MS/MS cihazı ile belirlenmiştir. Ayrıca ekstratların antioksidan, sitotoksik etkileri ile enzim inhibisyon aktiviteleri (antihipertansif, AchE (asetilkolinesteraz), BchE (bütilkolinesteraz), elastaz, tirozinaz, kollajenaz ve üreaz) belirlenmiştir. LC-MS/MS sonuçlarına göre *Z. capitata* türünün çiçek, yaprak, dal, karışık ve kök ekstratlarında kinik asidin (sırasıyla, 25578, 5842, 25171, 14055, 10597 $\mu\text{g g}^{-1}$) diğer bileşenlerden daha yüksek içeriğe sahip olduğu tespit

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Anahtar Kelimeler

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Kinik asit

edilmiştir. Ayrıca rosmarinik asit ($17097 \mu\text{g g}^{-1}$), sinarozit (8432) ve hesperidin (8067) bileşiklerinin majör bileşenler olduğu bulunmuştur. DPPH, ABTS ve CUPRAC yöntemlerinde türün çiçek ekstresinin güçlü antioksidan aktivitesi (sırasıyla: IC_{50} : $37.18 \pm 1.36 \mu\text{g mL}^{-1}$; 9.89 ± 0.45 ; $A_{0.5}$: 16.27 ± 0.02) sergilediği görülmüştür. *Z. capitata* türünün yaprak ekstresinin HT-29 (kolon kanseri hücre hattı) üzerine (% canlılık: 9.26 ± 0.69) güçlü sitotoksik etki gösterdiği belirlenmiştir. Bütirilkinesteraz (BChE) enzim inhibisyon aktivitesinde türün kök kısmının (%inhibison: 40.56 ± 0.88) diğer kısımlarından daha yüksek aktivite sergilediği görülmüştür. *Z. capitata* türünün etanol ekstrelerinin asetilkolinesteraz, üreaz, tirozinaz, elastaz, kollajenaz ve antihipertansif enzim aktivitesi göstermediği veya düşük aktivite gösterdiği belirlenmiştir. Sonuç olarak, *Z. capitata* türünün çiçek ekstresinin incelenen parametreler açısından daha iyi sonuçlara sahip olduğu, yaprak ekstresinin ise kolon kanseri hücre hattı üzerindeki sitotoksik etkisinden dolayı türün ilaç endüstrisinde kullanılabilmesi için *in vitro* ve *in vivo* olarak detaylı araştırmalara tabi tutulması gerektiği düşünülmektedir.

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INTRODUCTION

Plants are the starting point of the ancient medical system, which has been practiced for thousands of years and continues to provide novel treatments to the world. (Gurib-Fakim, 2006). Medicinal and aromatic plants have been used for centuries as spices and for the prevention of diseases. (Li, 2006; Christaki et al., 2012; Youssif et al., 2024). Medicinal plants are becoming increasingly important because of their potential to prevent and treat diseases. (Yu et al., 2021). Almost all cultures have used medicinal plants to treat diseases (Bozyel et al., 2019). Ensuring the safety, quality and efficacy of herbal products has become an important theme in today's world. (Singh, 2015).

The genus *Ziziphora*, belonging to the family Lamiaceae, is represented by five species and six taxa. In Turkey, it is grown in Western Anatolia, the Mediterranean Region and the Central and Eastern Anatolia regions. (Kaya & Dirmenci 2012; Satıl & Selvi, 2020). *Ziziphora* species are either annual or perennial herbs (Kaya & Dirmenci 2012). In the traditional medicinal practices of Turkey and Iran, *Ziziphora* species have been utilized for their sedative, stomach-soothing, aphrodisiac, anti-flatulence, and carminative properties (Sezik & Tümen, 1984; Kaya & Dirmenci, 2012; Selvi et al., 2015). In Turkey, species from this genus are referred to as 'Dağ Reyhanı', 'Filiskin otu', or 'Nane ruhu'. It is also known that the dried plant parts of this genus are used as herbal tea and spices. (Baytop, 1999; Kaya & Dirmenci, 2012; Selvi et al., 2015). *Z. capitata* species contains terpenoids, flavonoids, essential oils, and phenolic

acids (Ghazanfari et al., 2013; Youssif et al., 2024). It is also stated that this species has various pharmacological and cytotoxic effects on humans (Mohammadhosseini et al., 2016; Youssif et al., 2024). The essential oil composition of certain *Ziziphora* species from Iran has been studied before and was found to be abundant in oxygenated monoterpenes, including pulegone. The main constituents of the essential oil of the Iranian species *Z. clinopodioides* subsp. *rigida* were identified as piperitenone, thymol, pulegone and *p*-menth-3-en-8-ol (Ebrahimi et al., 2009).

In the literature review of *Z. capitata* species, it was found that germacrene D, (*Z*)- β -osimene, (*E*)- β -osimene, limonene, β -caryophyllene, hexadecanoic acid and bicyclogermacrene were the main components in its essential oil. Additionally, the antibacterial activity of *Z. capitata* oil was measured (Aghajani et al., 2008). Cytotoxic activity analysis of *Z. capitata* species against MCF-7 cell line and antioxidant, phenolic, and flavonoid analysis were performed (Mohammadhosseini et al., 2016; Youssif et al., 2024). There is no study on the inhibition of urease, AChE, BChE and tyrosinase enzymes by ethanol extract of *Z. capitata* species. In this sense, this study is the first of its kind.

In this study, ethanol extracts of flower, branch, mixed, root, and leaf parts of *Z. capitata* species were prepared. Phytochemical content analysis of the prepared extracts was performed by LC-MS/MS. The toxic effects of the extracts on PDF (healthy cell line) and cytotoxic effects on HT-29 and MCF-7 were determined and revealed. In addition, *in vitro*

antioxidant capacity (DPPH, ABTS, CUPRAC), acetylcholinesterase (AChE), butyrylcholinesterase (BChE), antihypertensive, elastase, collagenase, tyrosinase and urease enzyme inhibitory activities of the extracts were determined.

Table 1. Names of studied species, extract yield, and herbarium number

Çizelge 1. Çalışılan türlerin isimleri, ekstre verimi ve herbaryum numarası

Species Name	Code	Extract Yield %	Herbarium No
<i>Ziziphora capitata</i> flower	ZCC	5.42	
<i>Ziziphora capitata</i> leaf	ZCY	3.61	
<i>Ziziphora capitata</i> branch	ZCD	1.26	M.Fırat 32648 (VANF)
<i>Ziziphora capitata</i> mixed	ZCKA	6.32	
<i>Ziziphora capitata</i> root	ZCKO	1.18	

Extraction and LC-MS Analysis

Z. capitata species were separated into leaf, branch, flower, root and mixed parts. The separated parts were ground into powder with a grinder and 10 g of each type were weighed. Then, ethanol (50 mL, 3x24 hours) was added to the plant samples. After solvent evaporation processes, crude extracts were obtained (Akdeniz et al., 2021). The extracts were adjusted to final concentrations of 1000 µg mL⁻¹ prior to LC-MS/MS injection. The phytochemical composition of the examined plant species was evaluated using a previously established and validated LC-MS/MS method. In this method, 53 compounds, particularly found in natural products, with phenolic acid and flavonoid form, and 3 internal standards were analyzed (Yilmaz, 2020).

Total Flavonoid-Phenolic Content, Antioxidant and Cytotoxic Activity Analyses

Total phenolic (as pyrocatechol equivalent) and flavonoid contents of *Z. capitata* ethanol extracts (ZCC, ZCY, ZCD, ZCKA, and ZCKO) were calculated (as quercetin equivalent) (Slinkard & Singleton, 1977; Moreno et al., 2000). To evaluate the antioxidant activity of *Z. capitata* species ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts, DPPH (free radical scavenging), ABTS (cation radical scavenging) methods were used and the values were calculated as IC₅₀. Additionally, the extracts were evaluated using the CUPRAC (Copper (II) reduction capacity) method and the results were calculated as A_{0.5}. (Blois, 1958; Re et al., 1999; Apak et al., 2004; Tural et al., 2024). Butylated hydroxytoluene (BHT) and α-tocopherol were applied as references. Additionally, the method established by Mojarraba et al. (2013) was employed, with slight modifications, to evaluate the toxic and cytotoxic activities of the species ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts.

MATERIAL and METHOD

Plant Material

The species name, code, extract yield, and herbarium number of the used part of the *Ziziphora capitata* L. plant collected in Van province in 2016 are given in Table 1.

Enzyme Activities

Cholinesterase enzyme activity

To inhibit the enzymes acetylcholinesterase and butyrylcholinesterase, the method developed by Ellman et al. (1961) was used. In this method, galantamine was used as a reference when measuring enzyme inhibition.

Urease enzyme activity

Urease enzyme inhibition activity of ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts was determined using the method developed by Zahit et al. (2015). Thiourea was used as reference.

Tyrosinase enzyme activity

Tyrosinase enzyme inhibition activity of ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts was determined using the method developed by Hearing & Jiménez (1987). Kojic acid was used as a reference in this method.

Elastase and Collagenase enzyme activities

To determine the antiaging effects of the samples, elastase (Kraunsoe et al., 1999) and collagenase (Thiring et al., 2009) inhibitory activity determinations were performed. N-succinyl-(Ala)₃-nitroanilide and N-(3-[2-Furyl]acryloyl)-Leu-Gly-Pro-Ala were used as substrates in elastase and collagenase enzyme inhibitory activity determinations, respectively. Oleanolic acid and epicatechin gallate were used as standard references.

Hypertensive enzyme activity

The method described by Kwon et al. (2006) was used with minor variations. Lisinopril was used as standard.

RESULTS and DISCUSSION

LC-MS/MS analysis

According to the LC-MS/MS results of ethanol extracts

of *Z. capitata* species (ZCC, ZCY, ZCD, ZCKA, and ZCKO), the main components observed in all extracts were quinic acid (25578, 5842, 25171, 14055, 10597, $\mu\text{g g}^{-1}$, respectively), cynaroside (4349, 6371, 1417, 8432, 106), hesperidin (3246, 8067, 1554, 3016, ND) and rosmarinic acid (17097, 3663, 6950, 4179, 12013). It was determined that the compound with the highest

amount in all studied parts of the *Z. capitata* species was quinic acid. It is seen that all extracts studied contain caffeic acid and the highest amount belongs to ZCKO extract (4088). It was concluded that the leaf extract of *Z. capitata* (ZCY) contains higher hesperidin (8067) than other extracts (Table 2 and Figure 1 and 2).

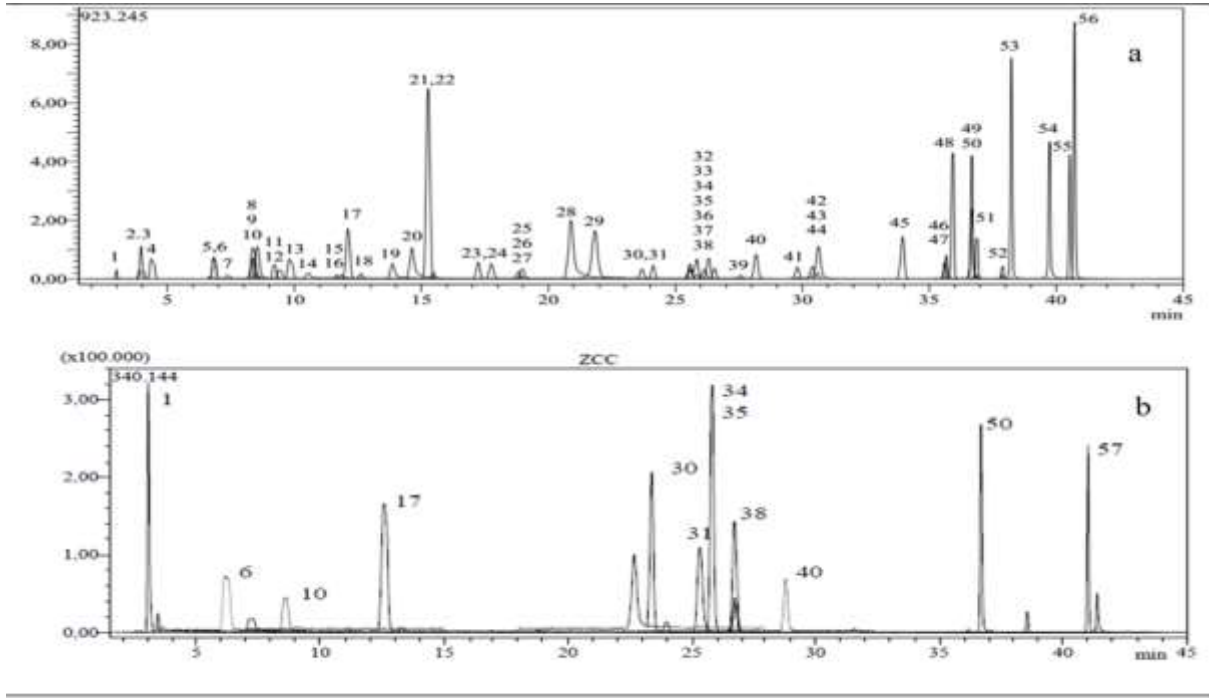


Figure 1. a) TIC chromatogram of the standards mixture analyzed by the LC-MS/MS ($1 \mu\text{g mL}^{-1}$). b) LC-MS/MS chromatogram of ethanol extract of flower part of *Z. capitata* species

Şekil 1. a) LC-MS/MS ile analiz edilen standart karışımının TİK kromatogramı ($1 \mu\text{g mL}^{-1}$). b) *Z. capitata* türünün çiçek kısmının etanol özütünün LC-MS/MS kromatogramı

There is no research on the phytochemical content of the *Z. capitata* species in the literature. However, some components found in the species were reported to be determined qualitatively by UPLC-QTOF-MS/MS (Youssif et al., 2024). Ahmedi et al. (2021) identified 44 compounds following LC-MS examination of the ethanol extract from the *Z. clinopodioides* Lam. species. Taheri et al. (2023) identified the main constituents of *Z. clinopodioides* as quercetin, rutin and apigenin (16738.85, 15004.45, $106.25 \mu\text{g g}^{-1}$, respectively). Ozkan et al. (2020) also conducted a

study in which it was determined that the ethanol extracts of the root and aerial parts of the *Z. clinopodioides* species contained high amounts of quinic acid (14721.04 ± 120.71 , $9020.51 \pm 73.97 \mu\text{g g}^{-1}$, respectively), malic acid (2179.04 ± 24.62 , $1972.95 \pm 22.29 \mu\text{g g}^{-1}$) and rhoifolin (3593.31 ± 338.13 , $1044.74 \pm 98.31 \mu\text{g g}^{-1}$), which is parallel to this study. Compared with previous studies, the major compounds of *Z. capitata* appear to be partially different from *Ziziphora clinopodioides*.

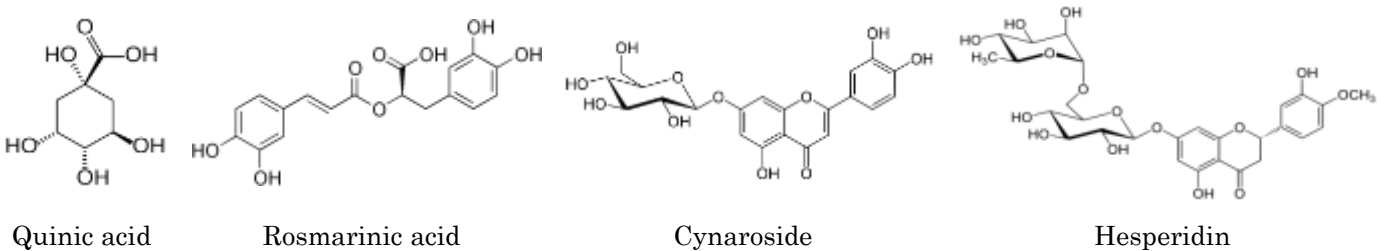


Figure 2. Major components of the species

Şekil 2. Türün major bileşikleri

Table 2. Phenolic and flavonoid contents of the studied species by LC-MS (μg analyte g^{-1} extract)
Çizelge 2. Çalışılan türlerinin LC-MS ile fenolik ve flavonoid içerikleri (μg analit g^{-1} ekstre)

No	Analytes	Retention time (min)	Parent ion (m/z) ^a	MS ² (Collision energy) ^b	Quantification (μg analyte g^{-1} extract) ^c				
					ZCC	ZCY	ZCD	ZCKA	ZCKO
1	Quinic acid	3.0	190.8	93.0	25578	5842	25171	14055	10597
6	Protocatechuic acid	6.8	152.8	108.0	1132	894	1395	1436	1879
9	Chlorogenic acid	8.4	353.0	85.0	ND	ND	ND	ND	157
10	Protocatechuic aldehyde	8.5	137.2	92.0	576	655	514	502	1201
17	Caffeic acid	12.1	179.0	134.0	1580	1362	2352	1692	4088
19	Vanillin	13.9	153.1	125.0	ND	ND	79	ND	60
24	<i>p</i> -Coumaric acid	17.8	163.0	93.0	130	167	76	407	65
28	Coumarin	20.9	146.9	103.1	10	ND	12	ND	ND
29	Salicylic acid	21.8	137.2	65.0	525	113	526	387	279
30	Cynaroside	23.7	447.0	284.0	4349	6371	1417	8432	106
33	Rutin	25.6	608.9	301.0	2605	5774	1144	2293	ND
34	isoquercitrin	25.6	463.0	271.0	1733	1842	1107	1443	ND
35	Hesperidin	25.8	611.2	449.0	3246	8067	1554	3016	ND
38	Rosmarinic acid	26.6	359.0	197.0	17097	3663	6950	4179	12013
40	Cosmosiin	28.2	431.0	269.0	714	847	147	1642	86
42	Astragalın	30.4	447.0	255.0	229	249	140	303	ND
47	Quercetin	35.7	301.0	272.9	121	79	122	85	ND
50	Luteolin	36.7	284.8	151.0/175.0	651	827	162	1705	61
52	Kaempferol	37.9	285.0	239.0	ND	ND	ND	ND	19
53	Apigenin	38.2	268.8	151.0/149.0	49	13	12	108	50
55	Chrysin	40.5	252.8	145.0/119.0	2	ND	27	10	156
56	Acacetin	40.7	283.0	239.0	242	238	207	694	4320

^aParent ion (m/z): Molecular ions of standards (mass-to-charge ratio). ^bMS² (CE): MRM fragments for the related molecular ions (CE refers to related collision energies of the fragment ions). ^cValues in $\mu\text{g g}^{-1}$ ($w w^{-1}$) of plant ethanol extract. ^dIS: Internal standard. ND: not detected. Numbers on the far left raw indicate the standard phytochemical compounds. (Components that are working but not detected: Fumaric acid, Aconitic acid, Gallic acid, Epigallocatechin, Catechin, Gentisic acid, Tannic acid, Epigallocatechin gallate, 1,5-Dicaffeoylquinic acid, 4-OH Benzoic acid, Epicatechin, Vanillic acid, Syringic acid, Syringic aldehyde, Daidzin, Epicatechingallate, Piceid, Ferulic acid-D3-ISd, Ferulic acid, Sinapic acid, Miquelianin, Rutin-D3-ISd, σ -Coumaric acid, Genistin, Ellagic acid, Quercitrin, Nicotiflorin, Fisetin, Daidzein, Quercetin-D3-ISd, Naringenin, Hesperedin, Genistein, Amentoflavone)

Total Flavonoid-Phenolic Content Results

When the total phenolic contents of ZCC, ZCY, ZCD, ZCKA, and ZCKO extracts of *Z. capitata* species were examined, it was determined quantitatively as ZCKO>ZCC>ZCY>ZCD>ZCKA (63.49±3.00, 51.98±1.82, 46.43±0.52, 40.08±1.82 and 33.93±0.42 μg Pes mg^{-1} extract, respectively). It is possible to rank the total flavonoid content as ZCKA>ZCY>ZCC>ZCD>ZCKO (45.14±0.32, 33.14±0.89, 16.86±0.36, 10.57±0.00, and 8.14±0.06, μg QEs mg^{-1} extract, respectively). It was found that the extract from the root part of the species had the highest total phenolic content (ZCKO), while the mixed part of the species (ZCKA) had the highest total flavonoid content (Table 3). In the study by Youssif et al. (2024), the quantitative analysis results of various active components, including phenolics and flavonoids, of the *Z. capitata* species were determined as 180.10±0.6 mg g^{-1} (gallic acid equivalent) and 40±0.8 mg g^{-1} (quercetin equivalent), respectively. It is evident that this study is parallel to the present study in terms of total flavonoid amount.

Antioxidant Activity Results

The results indicated that all extracts showed substantial antioxidant activity in each of the three methods. In the DPPH free radical scavenging method, all extracts showed significant antioxidant activity relative to BHT as a standard, with the ZCC extract (IC₅₀: 37.18±1.36 $\mu\text{g mL}^{-1}$) displaying the best activity. In the ABTS cation radical method, it was found that all extracts showed strong antioxidant activity, and the ZCKO (IC₅₀: 8.00±0.07 $\mu\text{g mL}^{-1}$) extract showed stronger activity. According to the CUPRAC copper reduction method, all extracts were found to exhibit strong antioxidant effects, and the ZCC (A_{0.5}: 16.27±0.02 $\mu\text{g mL}^{-1}$) extract had stronger activity (Table 3).

The antioxidant capacity of *Z. capitata* (IC₅₀: 206.6±1.3 $\mu\text{g mL}^{-1}$) was measured by the DPPH method in the study conducted by Mohammadhosseini et al. (2016). In the study conducted by Abad and Nadaf (2023), the essential oil of the *Ziziphora persica* Bunge was determined to have an IC₅₀ of 34.20±1.32 $\mu\text{g mL}^{-1}$

according to the DPPH method. When compared with previous studies, it was determined that *Z. capitata* species showed similarly high antioxidant activity.

Cytotoxic Activity

The toxic effects of ZCC, ZCY, ZCD, ZCKA, and ZCKO ethanol extracts of the species were studied against, as well as their cytotoxic effects against HT-29 and MCF-7. The percentage viability values of *Z. capitata* species extracts were determined at a 200 µg mL⁻¹ concentration. It was determined that all extracts did not have toxic effects on PDF cell lines. ZCY extract showed a very high cytotoxic effect on the HT-29 cell

line (viability %: 9.26±0.69). It was also found that ZCC extract had a good cytotoxic effect against the MCF-7 cell line (viability %: 47.72±0.81) (Table 3).

In the literature, in the study conducted by Youssif et al. (2024), hexane (46.7±1.68 µg mL⁻¹), chloroform (50.3±1.79 µg mL⁻¹), ethyl acetate (108±3.18 µg mL⁻¹), 95% ethanol (377±9.72 µg mL⁻¹), and water (407±11.28 µg mL⁻¹) extracts of *Z. capitata* species showed moderate cytotoxic activity against MCF-7 cell line. When the current study was compared with the previous study, moderate cytotoxic activity against MCF-7 was detected in the samples in both studies.

Table 3. Total phenolic, flavonoid, antioxidant and cytotoxic activity results of the studied extracts

Çizelge 3. Çalışılan ekstrelerinin toplam fenolik, flavonoid, antioksidan ve sitotoksik aktivite sonuçları

Sample s	Phenolic Content (µg Pes mg ⁻¹ extract) ^b	Flavonoid Content (µg QEs mg ⁻¹ extract) ^c	IC ₅₀ (µg mL ⁻¹) ^d		A _{0.5}		Vitality (%) ^e	
			DPPH Free Radical	ABTS Cation Radical	CUPRAC	HT29	MCF7	PDF
ZCC	51.98±1.82	16.86±0.36	37.18±1.36	9.89±0.45	16.27±0.02	232.61±2.77	47.72±0.81	126.65±0.95
ZCY	46.43±0.52	33.14±0.89	48.43±0.94	13.83±0.16	27.17±0.03	9.26±0.69	116.19±2.85	95.00±2.75
ZCD	40.08±1.82	10.57±0.00	60.91±1.25	23.34±0.26	26.16±0.02	207.60±0.94	90.71±1.37	89.98±3.81
ZCKA	33.93±0.42	45.14±0.32	54.52±1.24	15.55±0.25	22.84±0.01	200.25±6.48	73.93±0.72	89.33±3.59
ZCKO	63.49±3.00	8.14±0.06	37.40±0.98	8.00±0.07	23.14±0.03	245.42±1.00	73.72±3.38	105.09±0.65
α-TOC	-	-	13.10±0.52	10.48±0.63	14.49±0.11	-	-	-
BHT	-	-	62.15±0.35	13.62±0.28	7.69±0.01	-	-	-

^aValues are given as the average and standard deviation of 3 parallel measurements. ^bPyrocatechol equivalent phenolic content. (y = 0.0408(µg) + 0.0383 (r²: 0.9951)). ^cQuercetin equivalent flavonoid content. (y = 0.0355 (µg) + 0.0673 (r²: 0.9975)). ^dResults are given as IC₅₀ values. ^evitality (%) values at 200 ppm concentration. ZCC: *Z. capitata* flower, ZCY: *Z. capitata* leaf, ZCD: *Z. capitata* branch, ZCKA: *Z. capitata* mixed, ZCKO: *Z. capitata* root.

Enzyme Inhibition Activity

AChE, BChE, hypertensive, urease, collagenase, tyrosinase, and elastase enzyme inhibition activities of ZCC, ZCY, ZCD, ZCKA, and ZCKO ethanol extracts of the species were tested (Table 4). When the results were examined, it was found that the extracts were

inactive in the AChE enzyme inhibition test, all extracts were active in the BChE enzyme inhibition test, and ZCKA, ZCKO, and ZCY extracts (40.25±1.62, 40.56±0.88, and 40.25±1.62, respectively) showed moderate activity. The extracts were found to exhibit low or no inhibitory activity against urease, tyrosinase, elastase, collagenase, and hypertensive enzymes.

Table 4. Anticholinesterase, urease, tyrosinase, elastase, collagenase, and antihypertensive enzyme activity results of the studied species

Çizelge 4. Çalışılan türlerinin antikolinesteraz, üreaz, tirozinaz, elastaz, kolajenaz ve antihipertansif enzim aktivite sonuçları

Samples	Inhibition (%) ^a						
	AChE	BChE	Urease	Tyrosinase	Elastase	Collagenase	ACE
ZCC	5.07±0.97	24.87±0.73	13.55±0.69	17.25±0.16	AD	8.79±0.12	AD
ZCY	AD	40.25±1.62	AD	4.71±0.31	AD	9.63±0.08	AD
ZCD	AD	20.09±0.73	AD	AD	AD	7.45±0.05	AD
ZCKA	AD	40.25±1.62	AD	14.25±0.14	AD	7.28±0.06	AD
ZCKO	AD	40.56±0.88	AD	AD	AD	10.47±0.12	AD
Galantamine ^b	89.12±0.64	76.10±0.23	-	-	-	-	-
Thiourea ^b	-	-	94.64±0.16	-	-	-	-
Kojic acid ^b	-	-	-	91.64±0.23	-	-	-
Oleanolic acid ^b	-	-	-	-	44.32±0.20	-	-
Epicatechin ^b gallate ^b	-	-	-	-	-	43.80±0.12	-
Lisinopril ^b	-	-	-	-	-	-	97.68±0.42

^aValues are given as the mean and standard deviation of 3 parallel measurements (200 µg mL⁻¹). ^bStandard item. AD: Inactive. ZCC: *Z. capitata* flower, ZCY: *Z. capitata* leaf, ZCD: *Z. capitata* branch, ZCKA: *Z. capitata* mixed, ZCKO: *Z. capitata* root.

No studies were found in the literature regarding elastase, AChE, BChE, hypertensive, collagenase, tyrosinase, and urease enzyme inhibition activities of *Z. capitata*. This study is the first of its kind in this regard. However, when we look at the studies on other species belonging to the *Ziziphora* genus, in the study conducted by Ozkan et al., (2020), AChE, BChE, tyrosinase and urease enzyme inhibition activities of ethanol extracts of the aerial and root parts of the *Z. clinopodioides* species were determined and it was emphasized that only the extract of the aerial part of the species exhibited low activity in the tyrosinase enzyme inhibition test (inhibition %: 8.60 ± 0.87) and the extracts were not active in other enzymes. Sarıkurkcu et al. (2019) measured the tyrosinase enzyme inhibition activities of ethyl acetate (IC_{50} : 1.40 ± 0.06 mg mL⁻¹), methanol (1.25 ± 0.01), and water (2.71 ± 0.42) extracts of *Z. taurica* subsp. *cleonioides*. In another study conducted by Tomczyk et al. (2019), tyrosinase inhibition activities of ethyl acetate (IC_{50} : 1.37 ± 0.07 mg mL⁻¹), methanol (1.46 ± 0.06), and water (2.29 ± 0.13) extracts of *Z. taurica* subsp. *taurica* species were determined. When the results were examined, it was determined that the butyrylcholinesterase and tyrosinase inhibition activity of the *Z. capitata* species differed compared to other species.

CONCLUSION

This study involved the preparation of ethanol extracts from the flower, leaf, branch, mixed, and root components of the *Z. capitata* species, with their contents analyzed using LC-MS/MS and compared across 53 phytochemicals. The total phenolic and flavonoid contents of all extracts, cytotoxic activity, antioxidant capacities measured by DPPH, ABTS, and CUPRAC techniques, as well as the inhibition rates of hypertensive, urease, AChE, BChE, tyrosinase, elastase, and collagenase enzymes were assessed and compared. The LC-MS/MS analysis of *Z. capitata* revealed quinic acid, cynaroside, hesperidin, and rosmarinic acid as the predominant constituents in all extracts. The flower extract of *Z. capitata* species exhibited greater quantities of these components compared to other extracts. The total phenolic content of the root extract of the examined species was found to be superior to that of other extracts. The overall flavonoid concentration was greater in the mixed extract. All extracts from the *Z. capitata* species exhibited significant antioxidant activity, with the flower extract demonstrating the highest level of activity among them. *Z. capitata* leaf extract exhibited significant cytotoxicity against HT-29, although other extracts shown no cytotoxic impact on this cell line. The flower extract exhibited moderate cytotoxic activity against MCF-7, while the mixed and root extracts also shown moderate cytotoxic activity. Upon evaluation of enzyme inhibitory activity, it was

ascertained that only the flower extract shown activity against AChE and urease enzymes, exhibiting moderate activity. The BChE enzyme inhibition assay revealed that combined root and leaf extracts had moderate inhibitory efficacy. The flower extract exhibits superior activity compared to other extracts in the tyrosinase enzyme inhibition assay.

Consequently, it turned out that the flower extract of the *Z. capitata* species exhibited superior chemical composition and biological activity. Furthermore, given that the *Z. capitata* leaf extract exhibited a very high cytotoxic effect against the HT-29 cell line, the authors suggest that the *Z. capitata* species warrants further comprehensive *in vitro* and *in vivo* investigations for potential incorporation into the pharmaceutical sector.

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Contribution Rate Statement Summary of Researchers

Plant material: A.E, M.F.; Experimental studies: S.Y., M.V.C, I.Y., M.A.Y., E.C.K., M.C.; Data compilation and article writing: S.Y., M.C., A.E.

Conflict of Interest

The authors of the articles declare that they have no conflict of interest.

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