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Investigation of Flower, Leaf, and Stem Parts of Three *Gundelia* Species Growth in the Eastern Anatolia Region of Türkiye from a Biochemical Perspective

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ABSTRACT

13 species of *Gundelia* species plants are endemic in Türkiye and are frequently used by the public for both nutrition and medicinal objectives. In this study, three parts (flower, leaf, and stem) of three species of Gundelia (*G.dersim*, *G.glabra*, and *G.munzurensis*) were biochemically investigated. DPPH and ABTS analyses for antioxidant activity were performed on methanol extracts of plant parts. As a result of both analyses in parallel, the flower part of *G.munzurensis* had the highest %Inhibition value (DPPH:91.85±0.78; ABTS:97.38±0) (P<0.001). In total phenolic (29.09 mg GAE/g) and total flavonoid (5.59 mg CE/g) measurements, the highest amount was found statistically quite significant (P<0.001) in the leaf part of *G.munzurensis*. Gallic, vanillic, ferulic, romantic, and cinnamic acids, which are phenolic acids and of the flavonoids, rutin, catechin, naringin, and naringenin, were determined in all plant parts. Vitamin D3, alpha-tocopherol, ergosterol, stigmasterol, and beta-sterol were detected in parts of all plant species. While palmitic (16:0), palmitoleic (16:1), stearic (18:0), oleic (18:1), linoleic (18:2), and linolenic (18:3) fatty acids as the main fatty acids were detected in all parts of the three Gundelia species, lauric (12:0), tridecanoic (13:0), myristic (14:0), pentadecanoic (15:0), cis-10-pentadecanoic (15:1), heptadecanoic (17:0), cis-10-heptadecanoic (17:1), and eicosanoid (21:0) acids were also identified at different concentrations. Considering the biochemical properties of *Gundelia* species, it shows that they can be consumed as food and also used pharmacologically.

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Türkiye'nin Doğu Anadolu Bölgesinde Yetişen Üç *Gundelia* Türünün Çiçek, Yaprak ve Gövde Kısımlarının Biyokimyasal Açıdan İncelenmesi

ÖZET

Türkiye'de yayılış gösteren *Gundelia* L. cinsine ait 13 takson endemik olup halk tarafından hem beslenme hem de tıbbi amaçlarla sıklıkla kullanılmaktadır. Bu çalışmada *Gundelia*'ya ait üç türe (*G.dersim*, *G.glabra* ve *G.munzurensis*) ait üç kısım (çiçek, yaprak ve gövde) biyokimyasal olarak incelenmiştir. Antioksidan aktiviteye yönelik DPPH ve ABTS analizleri bitki parçalarının metanol ekstraktlarında yapıldı. Her iki analizin paralel olarak yapılması sonucunda *G.munzurensis*'in çiçek kısmı en yüksek % İnhibisyon değerine sahip olmuştur (DPPH:91.85±0.78; ABTS:97.38±0) (P<0.001). Toplam fenolik (29.09 mg GAE/g) ve toplam flavonoid (5.59 mg CE/g) ölçümlerinde en yüksek miktar *G.munzurensis*'in yaprak kısmında istatistiksel olarak oldukça anlamlı (P<0.001) bulunmuştur. Fenolik asitlerden gallik, vanilik, ferulik, rosmanirik ve sinnamik asitler ile flavonoidlerden rutin, kateşin, naringin ve naringenin tüm bitki

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Antioksidan aktivite
Yağ asitleri
Vitaminler
Fenolik asitler

kısımlarında tespit edilmiştir. Tüm bitki türlerinin bazı kısımlarında D3 vitamini, alfa-tokoferol, ergosterol, stigmasterol ve beta-sterol tespit edildi. Başlıca yağ asitleri palmitik (16:0), palmitoleik (16:1), stearik (18:0), oleik (18:1), linoleik (18:2) ve linolenik (18:3) yağ asitleridir. laurik (12:0), tridekanoik (13:0), miristik (14:0), pentadekanoik (15:0), cis-10-pentadekanoik (15:1), üç *Gundelia* türünün tüm kısımlarında tespit edildi. heptadekanoik (17:0), cis-10-heptadekanoik (17:1) ve henikosanoik (21:0) asitler de farklı konsantrasyonlarda tanımlandı. *Gundelia* türlerinin biyokimyasal özellikleri dikkate alındığında gıda olarak tüketilebildiği gibi farmakolojik olarak da kullanılabileceğini göstermektedir.

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INTRODUCTION

The genus *Gundelia* belongs to the *Asteraceae* family and is called "kenger" in Anatolia. With systematic studies, it has been found that the genus *Gundelia* is exemplified by 17 taxa in Türkiye and 13 of them are reported to be endemic (Ertas et al., 2021). *Gundelia* species are widely consumed by the public. It is also used for medical objectives for the treatment of many ailments such as liver, diabetes, heart, respiratory, and stomach pain (Ertas et al., 2021). *G. dersim* and *G. munzurensis* species discovered by Vitek et al. (2017) and *G. glabra* Mill species showed similar dispersion in the Tunceli province of Türkiye (Mikail et al., 2021). Comparative biochemical studies of these three species are important because the public frequently uses them for food and medical purposes and because they are similar. Besides, it was stated that species in ecologically different environments are important in point of biochemical studies (Mikail et al., 2021).

According to the literature review, *Gundelia tournefortii* L. was the most studied in detail among the *Gundelia* species (Coruh et al., 2007; Farhang et al., 2016; Hagi et al., 2011; Hajizadeh-Sharafabad et al., 2016; Khanzadeh et al., 2012; Matthäus and Özcan, 2011). At the same time, there were several studies on *G. dersim*, *G. glabra*, and *G. munzurensis* species (de la Luz Cádiz-Gurrea et al., 2020; Ertas et al., 2021; Mikail et al., 2021). However, in the present study, antioxidant properties, phenolic, and flavonoid contents, fatty acids, ADEK vitamins, and sterol profiles in methanol extracts of these three species were investigated separately. Therefore, this study comparatively examined the biochemical properties of the flower, leaf, and stem parts of these three types of plants (*G. dersim*, *G. glabra*, and *G. munzurensis*).

MATERIALS and METHODS

Plant materials and extraction

Among the plant materials, *G. munzurensis* (Figure 2) species were collected from Ovacık district (coordinates 39° 21′ 30.0024" N and 39° 12′ 57.9960" E) of Tunceli province, *G. dersim* and *G. glabra* (Figure 2) species were collected from Nizamiye district (coordinates 39° 10′ 51.9960" N and 39° 49′ 43.9968" E) of Tunceli province (Figure 1) in May 2021 (at the flowering period) by fieldwork. Species identification of the plants (Figure 2) was made by Prof. Dr. İsmail TÜRKOĞLU and Prof. Dr. Ebru YÜCE BABACAN. Population homogeneity was considered when plants were collected. Plants are divided into flower, leaf, and stem parts as shown in Figure 2.

The methanolic extract was used to identify the antioxidant properties and phenolic and flavonoid contents homogenized three plants' flower, leaf, and stem parts were homogenized separately with 85% methanol at a rate of 1:5 (w/v). Afterwards, the homogenates were centrifuged at 10000 rpm for 10 min (4°C) and the analysis was carried out by using the methanolic supernatant.

To determine the fatty acids and lipophilic molecule contents of the plants, each plant part was homogenized with HIP (n-hexane/isopropyl alcohol (3/2, v/v)) at a rate of 1:10 (w/v). After centrifugation at 10000 rpm for 10 min, the supernatants were analyzed.

Antioxidant activities

DPPH (Free radical extinguishing activity)

Free radical extinguishing activity (DPPH), was performed per the method specified by Brand-Williams et al. (1995). 25 mg/L α, α-Diphenyl-β-picrylhydrazyl (DPPH), as a free radical was dissolved in methanol. 4

ml of DPPH solution will be added to the glass test tubes, respectively, and then 50, 100, and 200 µL of all plant extracts were added and mixed by vortex, then incubated for 30 min at room temperature and in the dark. At the end of the incubation, mixture absorbances were read at 517 nm opposite a blank in the spectrophotometer. Decreased absorbance, the left behind amount of DPPH was determined as free radical extinguishing activity and the results were calculated according to the onlooking formula:

$\%Inhibition = [(Control_{ABS} - Sample_{ABS}) / Control_{ABS}] \times 100$

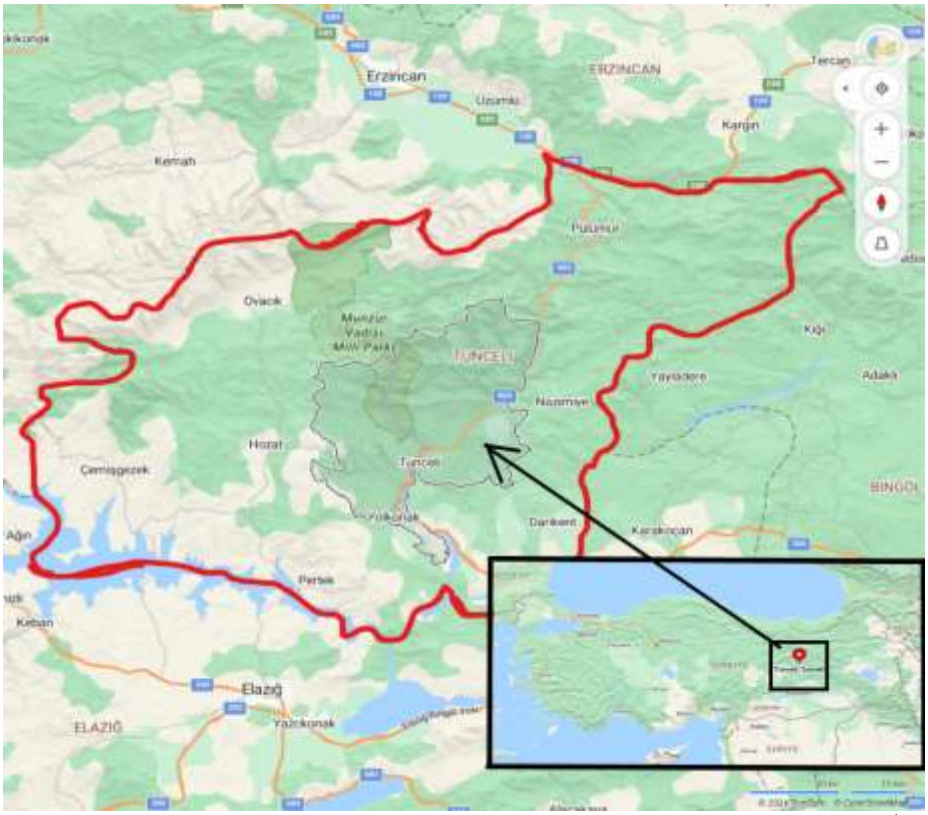

Figure 1. Tunceli province is the region where the *Gundelia* species used in the study are distributed (Bing Map, 2024)
Şekil 1. Tunceli ili, çalışmada kullanılan Gundelia türlerinin yayılış gösterdiği bölge (Bing Haritası, 2024)

Figure 2. *Gundelia dersim*, *Gundelia glabra*, and *Gundelia munzurensis* species and parts
Şekil 2. Gundelia dersim, Gundelia glabra ve Gundelia munzurensis türleri ve kısımları

ABTS (ABTS• radical elimination activity)

ABTS• radical elimination activity of plant samples Re et al. (1999) and Pellegrini et al. (2003) was performed according to these methods. After adding 2.45 mM potassium persulfate ($K_2S_2O_8$) to the final concentration of 7 mM 2,2-azinobis (3-ethylbenzothiazollin-6-sulfonic acid) (ABTS), the solution was retained in the dark at room temperature for 12-16 h. ABTS• radical cation solution formed because of the oxidation of potassium persulfate with

ABTS was brought to an absorbance density of 0.70 at a wavelength of 734 nm with ethanol. After this process, 50, 100, and 200 µL of plant extract was added to 2 ml of ABTS• radical cation and retained in the dark for 15 min. The absorbance was then evaluated at a wavelength of 734 nm. The number of ABTS• radicals eliminated by the extracts was calculated using the onlooking formula:

$\%Inhibition = [(Control_{ABS} - Sample_{ABS}) / Control_{ABS}] \times 100$

Phenolic components

The amount of total phenolic compounds

The amount of total phenolic compounds in plant extracts was measured by Singleton et al. (1999) method. Gallic acid was employed as standard. Briefly, 100 µL of plant extract was taken into a glass test tube and 0.5 mL of Folin-Ciocalteu reagent was suffixed to it. After 3 min, 3 mL of 2% Na_2CO_3 solution was suffixed to the samples and retained for 2 h with continuous mixing. At the end of this duration, the numbers of absorbances of the samples at 760 nm wavelength were read in the spectrophotometer, and results were calculated as gallic acid equivalent/g.

Determination of phenolic compounds by HPLC

Chromatographic analyses of phenolic acids were performed using a Prominence I LC-2030C3D plus compact HPLC system with some modifications. Chromatographic separations were realized on an Agilent Zorbax Eclipse XDB-C18 column and 4.6 mm x 150 mm, 3.5-µm particle size. The column was kept constant at 30±1°C during analysis. DAD signals for analytes were defined according to their spectrums acquired from the Lab Solutions LC/GC 5.91 Software. Suitable wavelengths for analysis were chosen between 254 nm and 333. Mobile phase A was 10 mM phosphoric acid and mobile phase B was methanol at an inflow ratio of 1 mL/min. The mobile phases flow program was as follows: 0-15 min (0-60% B), 15-20 min (60-80% B), 20-22 min (80-100% B), 22-27 min (100% B), and 27-32 min (0% B). The amount of samples injected into the system was 20 µL. Chromatographic peaks of the samples were compared with the spectra of standard references and sample quantity was determined (Dragovic-Uzelac et al., 2005; Gundogdu, 2013).

Flavonoid components

The number of total flavonoid compounds

The total amount of flavonoid substance was measured per the method applied by Kim et al. (2003). First, 0.3 mL of 5% sodium nitrite ($NaNO_2$) was added to 50 µL of plant extracts, and after 5 min 0.3 mL of 10% aluminum chloride ($AlCl_3$) was added. Thereafter, 2 mL of 1 M sodium hydroxide (NaOH) was added, and 2.4 mL of pure water was added and mixed by vortex. The absorbance values of the samples at 510 nm wavelength were read in the spectrophotometer and the last results were calculated as catechin equivalent/g.

Determination of flavonoid components by HPLC

For chromatographic analysis of flavonoids, a 5 µm inner diameter PREVAIL C18 (15x4.6 mm) reverse-phase column was used. A mixture of methanol/water/acetonitrile (46/46/8, v/v/v) having 1% acetic acid was employed as the mobile phase (Zu et al., 2006). This mobile phase was filtered through a 0.45 µm diameter membrane filter and then deaerated in the ultrasonication device before use. These flavonoids were measured by DAD following RHPLC separation using a wavelength of 265 nm for camphor, 254 nm for rutin, myricetin, morin, and quercetin, 280 nm for catechin and naringin, and myricetin, morin and quercetin, 306 nm for resveratrol. Adjusted at 1.0 mL/min and the injection value was 10 µL. The chromatographic peaks of the analysis were approved by comparison of reaction times and UV spectra of standard references. Quantification was performed by peak coupling employing the standard method and all chromatographic procedures were performed at 25°C.

Determination of ADEK vitamins and sterols by HPLC

To analyze fatty acids and lipophilic molecules, the supernatants obtained with hexane/isopropyl alcohol were taken into 5 mL test tubes, mixed by adding 5 mL of 10% KOH and retained at 85°C for 15 min. The tubes were brought to room temperature, pure water was suffixed and mixed. Unsaponifiable lipophilic molecules were extracted by adding 5 mL of hexane. Thereafter the hexane phase was evaporated using nitrogen gas. The remaining residue was dissolved in 1.0 mL (60% + 40%, v/v) acetonitrile/methanol solution and taken into autosampler vials. Analysis was accomplished with the Shimadzu brand HPLC device. An acetonitrile/methanol (60%+40%, v/v) solution was employed as the mobile phase for analysis and the mobile phase flow ratio was determined as 1 mL/min. DAD-UV detector was employed for vitamin A, D, E, and K analysis. A Nucleodur LC 18 (15x4.6 cm, 5 µm; MN USA) column was employed as the column, the detection wavelength of 326 nm for vit A and 202 nm for vit E, D, K, and phytosterols were employed (Katsanidis and Addis, 1999; Lopez-Cervantes et al., 2006).

Determination of fatty acid profile by GC

Isolation and methylation of fatty acids: Fatty acids were obtained by adding 10 mL of hexane/isopropanol solution to the plant parts. Then, 5 mL of the hexane phase of the samples was taken into test tubes and 5 mL of 2% methanolic sulfuric acid was added. This mixture was mixed with vortex and left at 55ºC for 12 h, and then brought to room temperature, 5 ml of 5%

NaCl solution was added and mixed with vortex. After the fatty acid methyl esters were received with 5 mL of n-hexane and treated with 5 mL of 2% potassium bicarbonate ($KHCO_3$) solution, the n-hexane phase was evaporated with nitrogen flow. Fatty acid methyl ester residues were dissolved in 1 mL of chloroform and received into autosampler vials.

Gas Chromatographic Analysis of Fatty Acids Methyl Esters: Fatty acids methyl esters were analyzed by SHIMADZU GC 17 gas chromatography and for this analysis, the SP™-2380 capillary GC arm (LxI.D. 30 mx0.25 mm, df 0.20 µm) (Supelco, Sigma, USA) was employed. During the analysis, the column temperaturdenime was retained at 120–220ºC, the injection temperature was 240ºC, the detector temperature was 280ºC, and the column temperature program was calibrated from 120ºC to 220ºC. Nitrogen gas was employed as bearer gas. First, by injecting mixtures of standard fatty acids methyl esters, the detention times of every fatty acid were determined, and then the programming was made and the analysis of fatty acids methyl esters of plant samples was performed (Christie, 1989).

Statistical Analysis

Statistical analysis of the obtained results was accomplished using the SPSS package program. ANOVA and LSD tests were applied to define the differences between the groups. The statistical significance limit was adopted as $P<0.05$. Data are given as mean ± SEM.

RESULTS

Antioxidant activities

DPPH (Free radical quenching activity)

DPPH results of flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurensis* species were measured at three different concentrations. DPPH results are given in Table 1 as %Inhibition. The 200 µL concentration of the flower part of *G. munzurensis* (91.85±0.78) had the highest %Inhibition DPPH, followed by the 200 µL concentration of the stem part of *G. munzurensis* (90.22±0.47). The lowest %Inhibition value was the 50 µL concentration of the stem part of *G. dersim* (4.47±0.28). When the concentration-dependent %Inhibition values of the flower, leaf, and stem parts of the three species were compared, the GdF50, GdF100, GdL50, GdL200, GdS50, GdS100, GgF200, GgS50, GgS100, and GmF200 groups were statistically more significant than all other groups ($P<0.001$). At the same time, the difference between GgF100&GmF50 and GgS200&GmS50 groups was statistically significant ($P<0.05$). Also, the difference between GmL100&GmL200 groups was statistically significant ($P<0.01$). Contrary to these results, the differences between GdF200&GdS200, GdL100&GgF50, GgL50&GmF100, GgL100&GmL100&GmS200, GgL200&GmL200, GmL50&GmS100 groups were statistically insignificant ($P>0.05$).

ABTS (ABTS• radical elimination activity)

The percentage Inhibition ABTS values of the flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurensis* species at three different concentrations are shown in Table 1. According to the findings, the highest %Inhibition ABTS value was measured in the groups of 200 µL concentration (97.38±0.00) of the flower part of *G. munzurensis*, 100 µL (97.27±0.11) and 200 µL concentration (97.09±0.06) of the stem part of *G. munzurensis*, respectively. The 50 µL concentration of the stem part of *G. dersim* (12.20±0.25) had the lowest %Inhibition value. When all groups were compared, the difference between the GdF50, GdF200, GdS50, GdS200, GgF100, and GgS50 groups and the other groups was statistically significant ($P<0.001$). In addition, the difference between GdL100&GmF50, GgS200&GmF100&GmL200, GmF200&GgL50, GmF200&GmL100, GmL100&GmS100&GmS200 groups was statistically significant ($P<0.01$). On the other hand, the difference between GdL100&GgS100, GdL200&GmS50, and GgL50&GmS200 groups was statistically significant ($P<0.05$).

Phenolic components

The total number of phenolic components

The total phenolic contents of flowers, leaves and stems of *G. dersim*, *G. glabra*, and *G. munzurensis* species were calculated as mg gallic acid equivalent/g and are shown in Figure 3. According to the findings, the group with the highest total phenolic content was the leaf part of *G. munzurensis* (29.09 mg/g), followed by the leaf part of *G. glabra* (20.03 mg GAE/g). The least amount of phenolic was measured in the flower part of *G. dersim* (1.89 mg GAE/g) and the stem part of *G. dersim* (2.10 mg GAE/g). The difference between the flower part of *G. dersim* and the stem part of *G. dersim* was statistically insignificant ($P>0.05$). On the other hand, the differences between all other groups were statistically significant ($P<0.001$).

Determination of phenolic compounds

Gallic, vanillic, ferulic, rosmaniric, cinnamic, and caffeic acids from phenolic acids were determined in the methanolic extracts of flowers, leaves, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurensis* species by HPLC device. The results are given in Table 2 as µg/g. Gallic and ferulic acids were determined in high amounts in the leaf parts of *G. munzurensis* and *G. glabra*. The highest amount of vanillic acid was measured in the stem part of *G. dersim*, rosmarinic acid in the leaf part of *G. glabra,* and cinnamic acid in

the leaf part of *G. munzurensis*. Caffeic acid was determined only in the stem part of *G. dersim* (29.50 µg/g). In other words, except for caffeic acid, other phenolic acids were determined almost in three parts of all plant species.

Table 1 ABTS and DPPH activities of methanolic extracts from *G. dersim*, *G. glabra,* and *G. munzurensis* species
Çizelge 1. G. dersim, G. glabra ve G. munzurensis türlerinden elde edilen metanolik ekstraktların ABTS ve DPPH aktiviteleri

Plant	Plant parts	Abbreviation	Concentration (µL)	ABTS (% Inhibition)	DPPH (% Inhibition)
G. dersim	Flower	GdF	50	15.81±0.41***	6.77±0.40***
			100	28.93±1.04	12.35±0.13***
			200	44.88±0.73***	21.82±0.61
	Leaf	GdL	50	24.95±0.32	17.71±0.20***
			100	49.91±0.63**	33.11±0.28
			200	72.39±1.72*	58.65±0.68***
	Stem	GdS	50	12.20±0.25***	4.47±0.28***
			100	23.90±0.78	9.21±0.15***
			200	39.53±0.63***	21.60±0.20
G. glabra	Flower	GgF	50	28.71±1.45	33.38±0.84
			100	55.88±1.37***	46.61±0.13*
			200	89.73±0.68	77.25±0.67***
	Leaf	GgL	50	95.12±0.66	83.27±0.35
			100	96.14±0.38	89.60±0.08
			200	91.07±1.74	87.61±0.20
	Stem	GgS	50	34.13±1.05***	25.37±0.41***
			100	52.02±0.29	37.18±0.87***
			200	86.16±0.52	61.44±0.85*
G. munzurensis	Flower	GmF	50	52.57±1.63	47.54±0.74*
			100	83.42±1.27**	83.31±0.55
			200	97.38±0.00	91.85±0.78
	Leaf	GmL	50	96.28±0.61	75.12±0.73
			100	94.72±0.23	89.55±0.55
			200	85.68±1.81	87.96±0.15
	Stem	GmS	50	70.35±1.30*	60.42±0.53*
			100	97.27±0.11	74.59±0.88
			200	97.09±0.06	90.22±0.47

***:P<0.001; **:P<0.01; *:P<0.005

Flavonoid components

1. The number of total flavonoid components

Total flavonoid amounts of flowers, leaves, and stems of *G. dersim*, *G. glabra*, and *G. munzurensis* species were calculated as mg catechin equivalent/g and are shown in Figure 3. According to the findings, the group with the highest total flavonoid content was the leaf part of *G. munzurensis* (5.59 mg CE/g), followed by the leaf part of *G. glabra* (4.45 mg CE/g). The least number of flavonoids was measured in the stem part of *G. dersim* (0.19 mg CE/g). The difference between the flower part of *G. dersim* and the stem part of *G. glabra* (0.43 mg CE/g and 0.40 mg CE/g, respectively) was statistically insignificant (P>0.05). In addition, the differences between the flower part of *G. dersim* and the stem part of *G. dersim*, the stem part of *G. dersim and the* stem part of *G. glabra*, and the flower part of *G. glabra and* the flower part of *G. munzurensis* were found to be statistically very significant (P<0.01).

2. Determination of flavonoid compounds

The flavonoids rutin, myricetin, catechin, naringin, naringenin, resveratrol, morin, quercetin, and kaempferol were determined by HPLC device in methanolic extracts of flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurensis* species. The results are shown in Table 2 as µg/g.

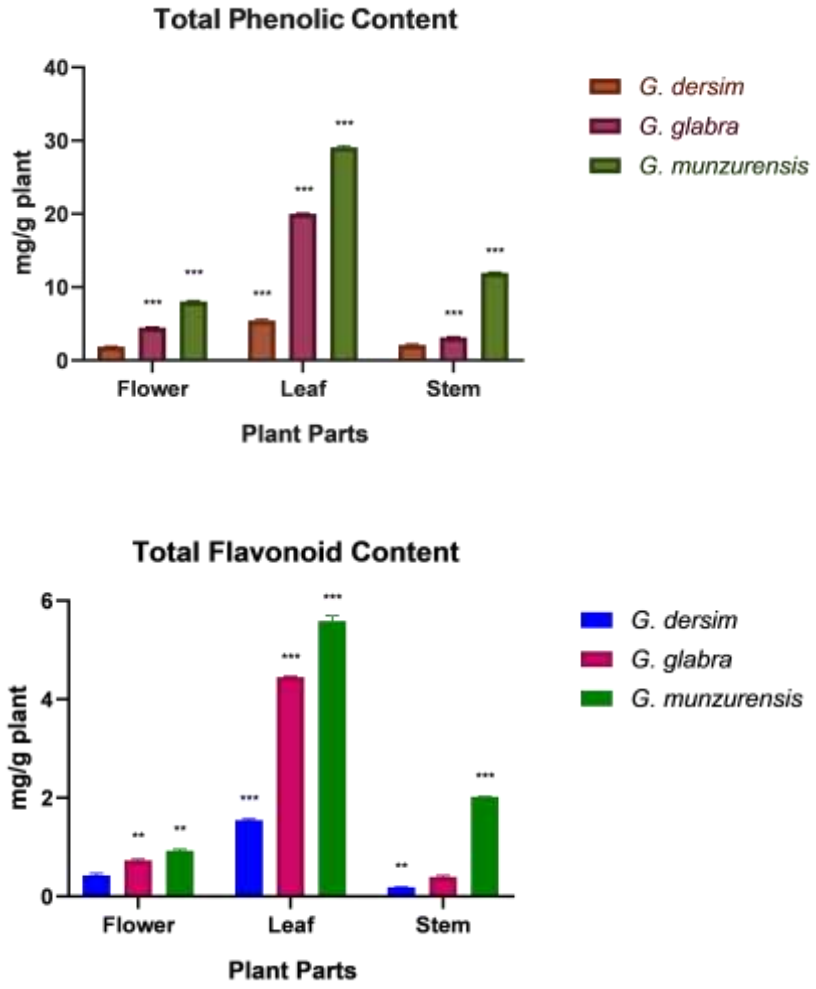

Figure 3. Total phenolic and flavonoid numbers of methanolic extracts obtained from *G. dersim*, *G. glabra*, and *G. munzurensis* species (***:P<0.001; **:P<0.01; *:P<0.005)

*Şekil 3. G. dersim, G. glabra ve G. munzurensis türlerinden elde edilen metanolik ekstraktların toplam fenolik ve flavonoid sayıları (***:P<0.001; **:P<0.01; *:P<0.005)*

Table 2. Phenolic acid and flavonoid contents of *G. dersim*, *G. glabra*, and *G. munzurensis* species

Çizelge 2. G. dersim, G. glabra ve G. munzurensis türlerinin fenolik asit ve flavonoid içerikleri

	G. dersim			***G. glabra***			***G. munzurensis***		
	Flower	Leaf	Stem	Flower	Leaf	Stem	Flower	Leaf	Stem
				Phenolic acids (µg/g)					
Gallic acid	236.00	362.00	177.50	476.50	1072.50	251.00	789.00	1413.00	361.00
Vanillic acid	70.00	104.00	115.00	42.50	45.00	28.50	72.00	47.50	22.50
Ferulic acid	17.50	21.50	10.50	40.00	2201.00	128.50	28.50	1694.00	93.50
Rosmaniric acid	3.50	10.00	3.00	6.50	23.50	11.00	5.50	9.00	8.50
Hcinnamic acid	4.50	2.50	2.00	3.00	2.00	2.50	1.00	13.50	2.00
Caffeic acid	*ND*	*ND*	29.50	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
				Flavonoids (µg/g)					
Rutin	6.25	156.50	16.75	139.00	1195.50	163.25	128.75	2619.75	1440.25
Myricetin	13.00	38.00	7.25	148.25	2630.00	*ND*	50.00	1930.75	319.25
Catechin	374.00	579.00	439.50	646.50	1109.25	397.75	74.00	84.00	409.00
Naringin	4.25	118.25	8.25	115.25	1084.75	126.75	114.75	2148.50	1187.00
Naringenin	153.50	1.00	0.50	148.50	10.50	1.50	2.00	4.50	1.50
Resveratrol	1.00	6.00	*ND*	10.75	246.25	21.75	6.25	182.75	29.00
Morin	*ND*	3.50	0.50	1.00	4.75	*ND*	*ND*	7.50	*ND*
Quercetin	*ND*	1.00	0.50	5.00	47.50	1.00	1.00	24.75	3.75
Kaempferol	*ND*	ND	0.50	90.50	858.25	54.25	7.50	110.00	8.50

ND: Not Detected

Rutin, myricetin, and naringin were observed in high amounts in the leaf parts of *G. munzurensis* and *G. glabra*. In addition, naringin was also high in the leaf part of *G. munzurensis*. The highest amount of catechin, resveratrol, and kaempferol was determined in the leaf part of *G. glabra*. Naringenin, on the other hand, was quite high in the flower parts of *G. dersim* and *G. glabra*. Myricetin was not observed in the stem of *G. glabra*, resveratrol was not observed in the stem of *G. dersim*, and quercetin was not observed in the lower part of *G. dersim*. Rutin, catechin, naringin, and naringenin have been identified in greater or lesser amounts in three parts of all plant species.

Determination of ADEK vitamins and sterols

Vitamins K and D, tocopherol (stigma and alpha), sterol (ergo, stigma, beta), retinol, and retinol acetate were determined by HPLC device in the flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurensis* species. The results are given in Table 3 as µg/g. According to the findings, D3, alpha-tocopherol, ergosterol, stigmasterol and beta-sterol were detected in more or fewer amounts in three different parts of three types of plants. While vitamin D3 was most abundant in the leaf part of *G. glabra* (57.00 µg/g), it was also high in the leaf part of *G. glabra* (46.75 µg/g). Ergo-sterol was found in very high amounts in the leaves of *G. munzurensis* (1141.25 µg/g). Similarly, stigma-sterol was quite high in the leaves of *G. dersim* (3143.75 µg/g) but very low in the leaves of *G. glabra* (13.75 µg/g). Beta-sterol was detected in the highest amount in the stem part of *G. munzurensis* (608.75 µg/g). Stigmatocopherol was not observed in the flower and stem parts of *G. glabra* yet was high in the leaf part (110.25 µg/g). Alpha-tocopherol was highest in the leaf part of *G. munzurensis* (167.11 µg/g). Retinol was detected in all plant parts except the stem part of *G. munzurensis,* and it was more abundant in the leaves of *G. glabra* (15.75 µg/g) than in the others.

Table 3. ADEK vitamin and sterol content of *G. Dersim, G. glabra* and *G. munzurensis* species (µg/g)
Çizelge 3. G. Dersim, G. glabra ve G. munzurensis türlerinin ADEK vitamini ve sterol içeriği (µg/g)

	G. dersim			***G. glabra***			***G. munzurensis***		
	Flower	Leaf	Stem	Flower	Leaf	Stem	Flower	Leaf	Stem
Vitamin K2	6.00	65.50	*ND*	*ND*	*ND*	*ND*	4.00	*ND*	2.50
δ-Tocopherol	4.75	13.00	2.25	*ND*	110.25	*ND*	2.00	101.50	7.25
Vitamin D2	25.25	21.00	19.00	*ND*	*ND*	*ND*	8.50	19.00	5.50
Vitamin D3	8.50	57.00	4.25	5.50	46.75	10.75	14.25	9.50	30.25
α-Tocopherol	8.25	32.75	1.00	6.25	92.00	1.50	3.50	167.11	35.34
Ergosterol	31.25	186.00	44.75	104.75	475.25	40.50	371.50	1141.25	30.75
Stigmasterol	282.75	3143.75	237.50	315.75	13.75	348.00	40.50	73.25	317.50
β-Sitosterol	95.00	95.50	87.75	164.75	187.25	173.00	174.75	338.50	608.75
Retinol	1.50	1.25	0.50	2.50	15.75	1.00	0.50	0.50	*ND*
Retinol acetate	8.50	57.50	2.50	19.00	1.75	*ND*	1.50	0.50	0.50
Vitamin K1	*ND*	141.25	*ND*	4.50	301.5	3.25	55.00	28.00	19.25

ND: Not Detected

Determination of fatty acid profile

The fatty acid profiles of the flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurensis* species defined by the GC device are shown in Table 4. Results are given as per cent concentration. These six essential fatty acids, called palmitic, palmitoleic, stearic, oleic, linoleic, and linolenic fatty acid essential fatty acids, are present in different amounts in all parts of three types of plants. Palmitic acid was mostly detected in the leaves of *G. dersim* (33.753%) and was also in high concentration in the leaves of *G. glabra*, and *G. munzurensis* (27.078, 30.228%, respectively). Palmitoleic acid, a monounsaturated fatty acid, was observed mostly in the stem part of *G. dersim* and least in the flower part of *G. munzurensis*. Stearic acid was determined mostly in the stem part of *G. munzurensis*. Oleic acid was mostly observed in the stem part of *G. glabra*, and linoleic acid was observed most in the flower part of *G. munzurensis*. Both fatty acids had the lowest concentration in the leaves of *G. munzurensis*. In contrast, the leaf part of *G. munzurensis* had the highest concentration of linolenic acid. In addition, high concentrations of linolenic acid were observed in the leaves of all three plant species. Apart from these fundamental fatty acids, lauric acid, tridecanoic, myristic acid, pentadecanoic, cis-10-pentadecanoic,

heptadecanoic, cis-10-heptadecanoic and eicosanoid acids were observed in various proportions in different parts of three plant species and the data are given in Table 4.

Table 4. Fatty acid contents of *G. dersim*, *G. glabra* and *G. munzurensis* species (%)
Çizelge 4. G. dersim, G. glabra ve G. munzurensis türlerinin yağ asidi içerikleri (%)

Fatty acids	*G. dersim*			*G. glabra*			*G. munzurensis*		
	Flower	Leaf	Stem	Flower	Leaf	Stem	Flower	Leaf	Stem
Lauric acid (12:0)	0.298	*ND*	*ND*	0.272	*ND*	*ND*	*ND*	*ND*	*ND*
Tridecanoic acid (13:0)	*ND*	0.452	*ND*	*ND*	*ND*	*ND*	*ND*	0.550	*ND*
Myristic acid (14:0)	0.921	1.026	0.921	3.663	0.847	0.755	*ND*	1.173	*ND*
Pentadecanoic acid (15:0)	*ND*	*ND*	*ND*	0.316	*ND*	*ND*	*ND*	*ND*	*ND*
Cis-10-pentadecanoic acid (15:1)	*ND*	1.443	*ND*	*ND*	1.590	*ND*	*ND*	1.353	*ND*
Palmitic acid (16:0)	26.172	33.753	22.095	17.605	27.078	17.282	11.039	30.228	17.212
Palmitoleic acid (16:1)	2.442	2.368	3.541	1.492	1.202	1.663	0.385	1.254	0.875
Heptadecanoic acid (17:0)	*ND*	*ND*	*ND*	0.202	*ND*	*ND*	*ND*	0.351	*ND*
Cis-10-heptadecanoic acid (17:1)	1.366	*ND*	1.110	0.545	*ND*	*ND*	0.247	0.888	*ND*
Stearic acid (18:0)	4.039	3.675	3.867	5.432	3.613	3.532	3.742	2.301	7.898
Oleic acid (18:1)	24.128	5.429	28.190	21.610	16.292	35.067	16.321	5.018	29.567
Linoleic acid (18:2)	29.053	11.346	31.420	39.170	19.014	33.654	65.514	9.052	41.132
Linolenic acid (18:3)	11.582	39.584	8.856	9.694	30.364	8.047	2.753	47.831	3.316
Henicosanoic acid (21:0)	*ND*	0.923	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*	*ND*
∑Saturated	31.43	39.829	26.883	27.49	31.538	21.569	14.781	34.603	25.11
∑Unsaturated	68.571	60.17	73.117	72.511	68.462	78.431	85.22	65.396	74.89
∑ MUFA	27.936	9.24	32.841	23.647	19.084	36.73	16.953	8.513	30.442
∑ PUFA	40.635	50.93	40.276	48.864	49.378	41.701	68.267	56.883	44.448

ND: Not Detected; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids

DISCUSSION

Extracts of flowers, leaves and stems of *Gundelia* species were obtained using methanol for better extraction of antioxidants and other endogenous compounds (Sultana et al., 2007). Antioxidant activities in methanolic extracts of plants were determined by DPPH and ABTS analysis. ABTS shows cation radical and DPPH a free radical scavenging activity. According to the findings, when *G. dersim*, *G. glabra*, and *G. munzurensis* species were compared in general, *G. munzurensis* showed the highest DPPH activity first. *G. glabra* took the second order and *G. dersim* took the third order. %Inhibition ABTS activity results showed parallelism with DPPH activity results. When the flower, leaf, and stem parts of all three species were examined separately, the highest DPPH and ABTS activities were observed in the leaf, stem, and flower parts of *G. munzurensis*. In the study of Ertas et al. (2021), disseminule ethanol extracts of seventeen *Gundelia* species were obtained and their DPPH and ABTS activities were examined. According to the results of the study, the highest DPPH and ABTS activity was observed in *G. colemerikensis*. In addition, similar to the results of this study, both DPPH and ABTS activities were found to be from high to low: *G. munzurensis*, *G. glabra*, and *G.dersim*. In another study examining the DPPH and ABTS activities of *G. dersim* and *G. glabra* extracts obtained by different extraction methods, it was stated that the infusion extract of *G. dersim* showed higher radical scavenging activity compared to the infusion extract of *G. glabra* (de la Luz Cádiz-Gurrea et al., 2020). Antioxidants are agents that inhibit radicals that damage macromolecules. Phenolic acids, polyphenols, and flavonoids found in plants are critical in terms of having antioxidant properties (Losso et al., 2007). There are reports that the phenolic components of plant extracts are related to their antioxidant activities (Aruoma et al., 2003; Škerget et al., 2005). When evaluated from this viewpoint, the total phenolic amounts and antioxidant activity values are consistent

with the findings of this study. In a study by Ertas et al. (2021), they found the total phenolic amount as Gm>Gg>Gd and the total flavonoid amount as Gd>Gm>Gg in ethanol extracts of plants. Although these results and total phenolic amounts in the current study were similar, total flavonoid amounts differed. This may be due to the difference in the solvent used to obtain the extract and the region where the plants are grown.

HPLC method is widely used for the identification of phenolic compounds in plant materials. It has also been stated that the extraction of these phenolic compounds is affected by their chemical structures, extraction method, particle sizes of the samples, storage time, and conditions (Naczk and Shahidi, 2004). Even though Ertas et al. (2021) detected rosmarinic acid and naringenin in *G. dersim*, *G. glabra*, and *G. munzurensis* species, they could not detect kaempferol. In the present study, kaempferol was found in each part of all other plants except the flower and leaf parts of *G. dersim*. Ferulic acid and myricetin have been reported in *G. glabra*, whereas rosmarinic acid, quercetin, and kaempferol have been reported in *G. glabra* and *G. dersim* species (de la Luz Cádiz-Gurrea et al., 2020). In the findings obtained, ferulic and rosmarinic acids were determined in the highest amount in the leaf part of *G. glabra* species (Table 2). In addition, hydroxycinnamic acid derivatives were detected in *G. tournefortii* L. (Haghi et al., 2011), *G. glabra* and *G. dersim* species (de la Luz Cádiz-Gurrea et al., 2020). *G. tournefortii* has been reported to be affluent in phenolic compounds and flavonoids (Asadi-Samani et al., 2013; Haghi et al., 2011; Nakatani et al., 2000). Besides, the high antioxidant properties of *G. tournefortii* have been associated with the flavonoids gallic acid and quercetin (Coruh et al., 2007). In line with this information, the results of the present study show similarities with the literature.

The hexane extracts of three different parts of *Gundelia* species were analyzed for vitamins by HPLC and for fatty acid by GC device analysis. In a study, the amount of beta-sterol in *Gundelia* species was found to be Gg>Gd>Gm from high to low (Ertas et al., 2021). In the current study, these results were found to be Gm>Gg>Gd. It has been reported that the biochemical analysis of plants may be affected depending on the ecological conditions in which the plant grows (stress, infection, photosynthesis rate, etc.) (Mikail et al., 2021). Matthäus and Özcan (2011) stated in their study that *G. tournefortii* L. has predominantly ß-sitosterol in the total sterol content, followed by stigma-sterol. Additionally, α-tocopherol was reported as the major tocopherol in the same study. In the current study results, the predominant sterol was stigmasterol.

Ertas et al. (2021) reported that oleic, linoleic, palmitic, and stearic acids are the primary fatty acids in *Gundelia* species. The same researchers stated that the ∑Unsaturated/∑Saturated ratio of *Gundelia* species is >1, and the plants are rich in unsaturated fatty acids. In parallel with these results, ∑the Unsaturated/∑Saturated ratios of three different parts of three plants were found to be >1 in the current study (Table 4). In a study investigating the fatty acid composition of *G. tournefortii* L. flower buds, linoleic acid was found the most, while oleic, palmitic, stearic, arachidic, and linolenic acids were identified (Matthäus and Özcan, 2011). In different studies on *G. tournefortii* L., linoleic, linolenic, and oleic acids were specified as the main fatty acids (Asghari et al., 2015; Khanzadeh et al., 2012; Mahmood et al., 2014). In a study investigating the fatty acid content of *G. rosea*, oleic and linoleic fatty acids were found as the main fatty acids (Dalar et al., 2019). Compared to the literature, the present study results show similarities in fatty acid compositions, even though the percentage values are different. It can be considered that the reason for these differences may be due to the various collection times, collection places, and ecological conditions of the plants.

CONCLUSION

Separate biochemical analyses of the flower, leaf, and stem parts of *G. dersim*, *G. glabra*, and *G. munzurensis* plants were performed and compared with each other. Considering the results of the study, *G. munzurensis* showed the highest antioxidant activity and *G.dersim* showed the lowest activity. Total phenolic and total flavonoid amounts attributed to high antioxidant activity also supported these results. *Gundelia* species contained high levels of sterols and tocopherol, vitamin K, vitamin D, and small amounts of retinol. In terms of fatty acid content, the ratio of unsaturated fatty acids in these three plants was also observed to be high.

Considering the natural antioxidant properties of *Gundelia* species and their vitamin and fatty acid compositions, it shows that they can be consumed as food and used pharmacologically due to their health benefits and easy accessibility.

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Contribution of the Authors as Summary

The authors declare the contribution of the authors is equal.

Statement of Conflict of Interest

The authors have declared no conflict of interest.

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