Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 30 (3), 703-713
Mustafa Kemal University Journal of Agricultural Sciences, 30 (3), 703-713
https://dergipark.org.tr/tr/pub/mkutbd
e-ISSN: 2667-7733

ARAŞTIRMA MAKALESİ **RESEARCH ARTICLE**

Antioxidant properties and phenolic components of extracts obtained from different parts of the *Iris kerneriana* (Çali süseni) plant

Iris kerneriana (Çalı süseni) bitkisinin farklı kısımlarından elde edilen ekstraktların antioksidan özellikleri ve fenolik bileşenleri

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ARTICLE INFO

Article history:
Recieved / Geliş: 01.05.2025
Accepted / Kabul: 29.07.2025

Keywords:
Antioxidant
Iris kerneriana
LC-MS/MS
Phenolic compound

Anahtar Kelimeler:
Antioksidan
Iris kerneriana
LC-MS/MS
Fenolik bileşik

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ABSTRACT

The antioxidant properties of different parts of the *Iris kerneriana* Asch. & Sint. ex Baker plant in different solvents will be revealed and their phenolic content will be determined. The information obtained about the plant as a result of the study will be pioneering for use in different sectors (food, cosmetics, medicine, etc.). Revealing the content of the plant; both the elucidation of the content of the plant and the detection of the presence of phenolic substances known to have positive effects on human health are preliminary studies for the use of the plant as a medicinal aromatic plant. In the research, phenolic compounds of extracts obtained from different parts of *I. kerneriana* plant in different solvents were determined and their antioxidant properties were analyzed. Three different parts of the *I. kerneira* plant were extracted with three different solvents. The plant was evaluated according to four different analysis methods according to parts and solvents. The highest detected compounds by LC-MS/MS were found to be phenolic compounds such as catechin (187.87 mg/kg), chlorogenic acid (116.07 mg/kg), caffeic acid (21.36 mg/kg) and vanillin (8.77 mg/kg). According to the results obtained from this study, the determination of the antioxidant capacity and phenolic substance content of the plant revealed that this plant may have significant potential in terms of pharmacology and in the food industry.

ÖZET

Iris kerneriana Asch. & Sint. ex Baker bitkisinin farklı kısımlarının farklı çözücülerdeki antioksidan özellikleri ortaya koyulacak ve fenolik içeriği belirlenmiştir. Çalışma sonucunda bitki hakkında elde edilen bilgiler farklı sektörlerde kullanılmak üzere (gıda, kozmatik, ilaç vb) öncü olacaktır. Bitkinin içeriğinin ortaya konması; hem bitkinin içeriğinin aydınlatılması hem de insan sağlığına pozitif etkilerinin olduğu bilinen fenolik maddelerin varlığının tespiti, bitkinin tıbbi aromatik bir bitki olarak kullanımı için ön çalışma niteliğindedir. Araştırmada, *I. kerneriana* bitkisinin farklıkısımlarından elde edilen ekstraktların farklı çözücülerdeki fenolik bileşikleri tespit edildi ve antioksidan özellikleri analiz edildi. *I. kerneira* bitkisinin üç farklı kısmı üç farklı çözücü ile ekstre edildi. Bitki kısımlarına ve çözücülere göre dört farklı analiz yöntemine göre değerlendirildi. LC-MS/MS analizleriyle en yüksek düzeyde tespit edilen bileşiklerin, kateşin (187.87 mg/kg), klorojenik asit (116.07 mg/kg), kafeik asit (21.36 mg/kg) ve vanilin (8.77 mg/kg) gibi fenolik bileşikler olduğu saptanmıştır. Bu çalışmadan elde edilen sonuçlara göre bitkinin antioksidan kapasitesi ve fenolik madde içeriğinin belirlenmesi, bu bitkinin farmakolojik açıdan ve gıda endüstrisinde önemli bir potansiyele sahip olabileceğini ortaya koymuştur.

Cite/Atıf Doğan, H., Kayır, Ö., Yaylacı, Ö.K., & Cömert, B. (2025). Antioxidant properties and phenolic components of extracts obtained from different parts of the *Iris kerneriana* (çali süseni) plant. *Mustafa Kemal Üniversitesi Tarım Bilimleri Dergisi, 30* (3), 703-713. https://doi.org/10.37908/mkutbd.1688669

INTRODUCTION

Before starting the culture studies of natural plants, it is important to characterize their functional compounds, determine their biological activities, and determine them as food additives (coloring, preservatives, odor, taste, etc.). The use of plants for medicine, food and cosmetic purposes began with the existence of man and has continued until today. Over time, the use of plants and the specific properties of each have been discovered by people for the purpose of treating common diseases and their treatment, and have been developed through positive or negative experiments (Baytop, 1999). Humans use plants for a variety of functions, protection, including nutrition, tools, warmth, and disease management. Furthermore, the significance of plants as natural resources in the field of medicine is of considerable importance. Many studies have indicated that plants have a wide range of biological activities covering anticancer, antibacterial, antioxidant, anti-inflammatory, antiproliferative, anti-aging, antiallergic, hepatoprotective and DNA protective roles (Sevindik et al., 2025).

I. kerneriana also known as the "Çalı süseni", it is a plant species with fragrant, yellow flowers that grows in Türkiye and belongs to the Iris genus. *I. kerneriana* named after the Austrian botanist Kerner. This is a small Iris with a stem about 15 to 30 cm long, bearing two yellow flowers with pointed, lanceolate blades towards the cascades. It is very similar to I. sintenisii except for the color of the flowers, the shape of the cascades, and the lack of sharp keeled spikes. *I. kerneriana* can be observed in open pine forests, dry grasslands and dry soils at altitudes of 300–2300 m above sea level (A Handbook of Garden Irises, 2009). A 2011 study identified volatile compounds such as longipenen, capric acid and hexadecanoic acid (Başer et al., 2011).

The genus Iris is a strong candidate derived from natural sources, with a broad spectrum of applications in the traditional cuisine of different countries worldwide. The substance's pleasant, sweet flavour has led to its utilisation in the aromatisation of a variety of products, including soft beverages, confectionary, chewing gum and bread flour, in multiple countries. Recent scientific research has indicated that the isolated compounds and crude extracts of this particular plant possess significant antioxidant and antimicrobial properties, especially with regard to food-poisoning bacteria and fungi. These findings underscore the potential of Iris based extracts to enhance the shelf life of food products and as flavouring agents (Khatib et al., 2022).

Phenolic compounds are bioactive natural products that have attracted considerable interest in the fields of pharmaceuticals and biomedicines. The reason for this increased attention is the bioactive functions of these compounds (Özderin, 2021). In recent decades, there has been a notable increase in research studies focusing on phenolic compounds for pharmaceutical and biomedical applications (Uçan Türkmen et al., 2024). One of these investigations is a study that provides an overview of the derivatives of caffeic acid and its general properties, including its biosynthesis, pharmacological and therapeutic effects and biological activities. In this rewiew, information on the medical and pharmacological effects of caffeic acid such as atherosclerotic, cardioprotective, immunomodulatory, hypertension, radiotherapy, neurodegeneration, neuroprotective, anxiety, vasoactive, dyslipidaemia and obesity is given (Yazar et al., 2025). This increased focus is due to the various beneficial effects these compounds have on human health. It is an irrefutable fact that phenolic compounds offer significant benefits and represent a promising source of value in the fields of pharmaceuticals and biomedicines. This is due to their high antioxidant capacity, as well as their anticancer, antimicrobial, antiviral and anti-inflammatory properties (Ge et al., 2020). Phenolic compounds have been shown to exert a significant impact on various physiological systems within the body. These include, but are not limited to, the immune system and defence mechanisms against pathogens. Furthermore, these compounds have been observed to influence memory, mood, behaviour, and cognitive function (Maheshwari & Sharma, 2023). Phenolic compounds have been demonstrated to possess the capacity to modulate gene expression through the regulation of epigenetic mechanisms, encompassing DNA methylation, histone modification, and microRNA expression (Číž et al., 2020). In the treatment and management of cancer, phenolics represent a class of plant-based compounds that have attracted considerable scientific interest.

They are found to effectively scavenge many non-biological superoxide radicals, nitrogen and chlorine species, free radicals, H2O2, and various ROS (Maheshwari & Sharma, 2023). Many phenolic compounds have anti-inflammatory properties. These compounds have been shown the secretion and production of pro-inflammatory cytokines, as well as the synthesis of reactive oxygen species (ROS) and nitric oxide (NO) (Číž et al., 2020). Phenolic compounds have demonstrated the capacity to impede the progression of numerous serious diseases, including cancer, Alzheimer's, and diabetes, amongst others. The underlying mechanisms of these beneficial effects are attributed primarily to the antioxidant and radical scavenging properties of these compounds, which have been shown to retard or prevent the oxidation of DNA, proteins, and lipids (Albuquerque et al., 2021).

The addition of artificial antioxidants to prevent free radicals is one of the most effective methods widely used. The oxidation stability of the added substance is also very important in this sense. Additives with high stability that are not affected by temperature changes should be used. Nowadays, there has been an increased tendency towards natural resources instead of artificial ones (Favaro et al., 2018). The present study investigates the antioxidant capacity and phenolic compound content of the *I. kerneriana* plant. Consequently, the study revealed the plant's significant potential in the domains of pharmacology and the food industry.

MATERIALS and METHODS

Plant material

I. kerneriana was collected from Çorum province and its villages. The plant was identified by Dr. Ömer Koray YAYLACI. Plant samples was collected in the region of Çorum with the approval of the Republic of Türkiye Ministry of Agriculture and Forestry.

Preparation of extracts for chemical analysis

Flower, stem and root parts of the plant were used in studies to extract the phenolic compound profile and determine the antioxidant capacity. Within the scope of the study, approximately 5 g were taken from the relevant part of the dried plant sample and crushed in a mortar with liquid nitrogen, and then weighed into 200 mg capped glass tubes. 10 mL of methanol/dichloromethane mixture (5:1 %v/v) was added and mixed. After being kept in an ultrasonic bath for 30 minutes twice, it was kept at +4 oC until analysis. The obtained extract was used in LC-MS/MS analysis for both antioxidant activity analyses and phenolic compound profiles.

Total phenolic content

Total phenolic compound determination was done with Folin-Ciocalteu reagent (Slinkard & Singleton, 1977). After adding 4.5 mL of distilled water to 100 µL of sample extract solution, 100 µL of Folin-Ciocalteu reagent was added. After waiting for 3 min., 300 µL of 2% sodium carbonate (Na2CO3) was added and mixed, then after waiting for 2 hours at room conditions, the absorbance at 760 nm was recorded on a spectrophotometer. A calibration curve was created with different concentrations of gallic acid used as a standard and the results were given as mg gallic acid equivalent phenolic compound.g-1 dry plant.

Ferric Ions (Fe3+) reducing antioxidant power (FRAP)

This analysis was done according to Elmastas, M., (Elmastaş et al., 2006) with some modifications of the Oyaizu method (Oyaizu, 1986). 40 µL of plant extract was taken and its volume was completed to 1.25 mL with phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of potassium ferricyanide [K3Fe(CN)6] (1%) was added. This mixture is incubated at 50˚C for 20 minutes. After incubation, trichloroacetic acid TCA (1.25 mL, 10%) and FeCl3 (0.25 mL, 0.1%) were added to the mixture. The absorbance of the final mixture was recorded at 700 nm and the absorbance values were calculated as µmol Trolox equivalent.g-1 dry plant using the Trolox (10–100 µmol.L-1) calibration chart.

Table 1. Calibration curve equations and regression values (R^2) of standard phenolic compounds

Çizelge 1. Standart fenolik bileşiklerin kalibrasyon eğrisi denklemleri ve regresyon değerleri (R^2)

No	Analyte	Parent ion (m/z)	MS/MS (CE)	Ionization Mode	Rt	Regression equation	R^2	RSD %	Linearity Range (mg/L)	LOD (mg/L)	LOQ (mg/L)
1	Pyrogallol	124.9	69.3 (20)-79.3 (23)	Neg	8.00	Y = 209926*X	0.9988	3.5	0.125-4.0	0.058	0.193
2	Gallic Acid	169.7	80.5 (25)-126.2 (16)	Neg	9.85	Y = 50092+348984*X	0.9977	4.5	0.125-4.0	0.061	0.203
3	Protocatechuic acid	155.0	65.4 (22)-93.2 (13)	Pos	11.77	Y = 35805.6+252538*X	0.9973	4.9	0.125-4.0	0.049	0.162
4	Protecatechuic aldehyde	136.9	92.3 (25)-108.2 (25)	Neg	12.64	Y = 828069+2.08203e+006*X	0.9963	2.2	0.125-4.0	0.026	0.087
5	Sesamol	137.2	108.3 (15)-109.3(14)	Neg	12.73	Y = 265762+920434*X	0.9979	2.1	0.125-4.0	0.031	0.103
6	Catechin	289,2	203.9 (22) – 245.7 (17)	Neg	12.95	Y = 294309+2.16741e+006*X	0.9972	3.1	0.125-4.0	0.010	0.032
7	Gentisic acid	153.7	109.5 (21)	Neg	12.99	Y = 230432*X	0.9493	2.3	0.125-4.0	0.013	0.043
8	Epicatechin	291.5	123.3 (15) – 139.3 (16)	Neg	14.38	Y = 698565+3.59164e+006*X	0.9956	2.5	0.125-4.0	0.003	0.006
9	Caffeic acid	179.7	135.2 (27) – 136.2 (18)	Neg	14.84	Y = 140481+917101*X	0.9959	2.5	0.125-4.0	0.042	0.058
10	Vanillin	150.9	92.3 (23)-136.1 (16)	Neg	15.36	Y = 29884.7+663215*X	0.9981	3.7	0.125-4.0	0.023	0.076
11	Syringic acid	183.07	77.3 (23)-123.2 (13)	Neg	15.76	Y = 4.66769e+007+1.06366e+008*X	0.9919	4.6	0.125-4.0	0.194	0.647
12	Syringic aldehyde	180.88	151.1 (24)-166.1 (16)	Neg	15.81	Y = 2.06035e+006*X	0.9828	4.1	0.125-4.0	0.177	0.590
13	Taxifolin	303.0	126.2 (23)-285.5 (15)	Neg	16.27	Y = -1840.83+4932.75*X	0.9553	3.8	0.125-4.0	0.001	0.005
14	P-Coumaric acid	163.9	94.3 (33) – 120.2 (17)	Neg	16.41	Y = 57037.7+333624*X	0.9974	3.5	0.125-4.0	0.069	0.109
15	Ferulic acid	195.0	89.4 (30)- 177.4 (7)	Pos	16.77	Y = 72958.8+555325*X	0.9985	4.5	0.125-4.0	0.063	0.118
16	Sinapic acid	223.0	149.1 (22)-164.1 (17)	Neg	16.95	Y = 16718.1*X	0.9797	4.1	0.125-4.0	0.211	0.703
17	Salicylic acid	137.1	65.5 (35)-93.3 (18)	Neg	17.19	Y = 2.41974e+006+1.61013e+007*X	0.9997	4.8	0.125-4.0	0.030	0.099
18	p_hydroxybenzoic acid	137.9	66.6 (38) – 94.6 (17)	Neg	17.28	Y = -300006+2.05222e+007*X	0.9981	4.7	0.125-4.0	0.243	0.809
19	Rosmarinic acid	359.2	134.3 (44) – 162.2 (20)	Neg	17.41	Y = 20533.4+313467*X	0.9991	2.6	0.125-4.0	0.003	0.005
20	Oleuropein	539.1	275.8 (22) – 377.5 (16)	Neg	17.59	Y = 2268.33+99450.9*X	0.9989	2.0	0.125-4.0	0.009	0.029
21	Rutin	609.37	300.6 (38) – 301.7 (34)	Neg	18.03	Y = 491933+3.52639e+006*X	0.9961	1.6	0.125-4.0	0.022	0.073
22	Rezveratrol	228.9	107.2 (22) – 135.1 (14)	Pos	18.14	Y = 2.8631e+006+1.2798e+007*X	0.9976	4.8	0.125-4.0	0.019	0.062
23	Ellagic Acid	301.7	284.8(30)-174.2(34)	Neg	19.16	Y = 227990+991531*X	0.9977	5.0	0.125-4.0	0.087	0.289
24	Cinnamic acid	147.8	78.4(22)-104.7(13)	Neg	19.44	Y = 221309*X	0.9871	4.1	0.125-4.0	0.104	0.347
25	Naringenin	273.0	147.1 (20) – 153.0 (24)	Pos	19.71	Y = 1.44729e+008*X	0.9936	4.8	0.125-4.0	0.005	0.017
26	Quercetin	301.0	152.1 (23) – 179.9 (20)	Neg	20.38	Y = 232619+8.40609e+006*X	0.9989	2.9	0.125-4.0	0.001	0.005
27	Kaempferol	286.9	153.0 (33) – 165.0 (28)	Pos	21.46	Y = 4.30138e+006+4.41591e+007*X	0.9925	4.4	0.125-4.0	0.188	0.447
28	Galangin	268.9	169.1(29)-171.1(31)	Neg	23.43	Y = 2.2251e+006*X	0.9977	5.0	0.125-4.0	0.034	0.113
29	Flavone	222.9	77.3(35)-121.2(26)	Pos	23.74	Y = 8.10507e+007+5.1598e+008*X	0.9957	1.7	0.125-4.0	0.037	0.124

Free radical scavenging activity (DPPH●)

Free radical (DPPH) scavenging activity was carried out according to the method applied by Blois, M.S et al. (BLOIS, 1958). 0.26 mM DPPH (2,2-diphenyl-1-picryl hydrazyl) solution was prepared in ethanol. After taking 40 µL from the stock solutions of plant extracts and adding 1 mL of DPPH (0.26 mM) solution, ethanol was added until the volume of the solution in the tube became 4 mL. Then, it was vortexed and incubated for 30 min. After the incubation process was completed, the absorbance of each sample was measured at 517 nm with a spectrophotometer. The results were calculated as IC50. The ability to scavenge the DPPH radical was calculated using the equation:

DPPH scavenging effect (%)=[(Ac-As)/Ac]x100

Ac is the absorbance of the control, and As is the absorbance in the presence of samples or standards.

Cation radical scavenging activity (ABTS+)

This analysis was performed according to the method applied by Re, R et al. (Re et al., 1999). According to this method, 2 mM ABTS (2,2'-Azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) solution prepared with phosphate buffer and 2.45 mM K2S2O8 (potassium persulfate) solution prepared with phosphate solution were mixed at a ratio of 1:2 and kept in the dark for 6 hours.

40 µL was taken from the stock solutions of the samples and phosphate buffer was added until the volume became 3 mL. Then, 1mL ABTS+ solution was added and the solution was mixed. After the solution mixture was incubated for 1 hour at room conditions, its absorbance was read at 734 nm in a spectrophotometer. The results were calculated as IC50.

Determination of phenolic substance components of plants by LC-MS/MS

Identification and quantification of phenolic compounds were performed using Dionex Ultimate 3000 model Thermo Scientific UHPLC instrument and tandem MS (TSQ Quantum Access Max) instrument (Kayir et al., 2023). For LC-MS/MS analysis of phenolic compounds in extracts of *I. kerneriana* plant, standard calibration curve graphs of 29 different phenolic compounds were drawn. Calibration curves along with R2 and line equations are given in Table 1.

Statistical analysis

The experimental data were analyzed using SPSS Statistics Version 22.0. We utilized the ANOVA test to identify any statistical differences in the results ($p < 0.05$). The results are presented as the mean ± standard deviation (n = 3).

RESULTS and DISCUSSIONS

Total phenolic content

The total phenolic content of the parts of the *I. kerneriana* plant is given as gallic acid equivalent (GAE). A high gallic acid equivalent (GAE) value indicates that the total phenolic content is high. The highest total phenolic content among all extracts was observed in the methanol extract of the stem part (10689 µmol GAE.g-1 dry plant). Additionally, it was found that the total phenolic content of the stem part was generally higher than the other parts in all extracts. It was observed that the total phenolic content in water and ethanol extracts of the root part was similar. These results are given in Figure 1a.

Ferric Ions (Fe3+) reducing antioxidant power (FRAP)

Antioxidant activities of water, methanol and ethanol extracts from different parts of I. kerneriana plant determined by FRAP method are given as µM Trolox equivalent (TEAC value). A high TEAC value indicates high antioxidant

activity. When the general evaluation of the stem, root and flower parts of the plant was made, the highest antioxidant capacity was determined in methanol extracts. In methanol extracts, the highest activity was determined in the stem part. These results are given in Figure 1b.

Table 2. Phenolic compound results (mg/kg) and standard deviations of plant extracts
Çizelge 2. Fenolik bileşik sonuçları (mg/kg) ve bitki özütlerinin standart sapmaları

		Ethanol ± sd	**Methanol ± sd**	**Water ± sd**
Gallic acid	Flower	<LOD	2.36^{a} ± 1.47	<LOD
	Stem	<LOD	<LOD	<LOD
	Root	6.20^{b} ± 1.46	9.01^{c} ± 0.58	$8.33^{b,c}$ ± 0.84
Protocatechuic acid	Flower	<LOD	<LOD	35.80^{b} ± 2.67
	Stem	<LOD	<LOD	<LOD
	Root	<LOD	<LOD	11.47^{a} ± 6.19
Protocatechuic aldehyde	Flower	<LOD	<LOD	<LOD
	Stem	<LOD	<LOD	<LOD
	Root	2.85^{b} ± 0.15	5.53^{c} ± 0.55	1.16^{a} ± 0.45
Catechin	Flower	39.19^{d} ± 2.10	$42.32^{d,e}$ ± 4.80	46.28^{e} ± 0.56
	Stem	185.98^{g} ± 9.00	187.87^{g} ± 6.88	116.53^{f} ± 10.08
	Root	14.85^{b} ± 0.22	25.01^{c} ± 0.61	7.79^{a} ± 0.26
Chlorogenic acid	Flower	20.10^{b} ± 2.94	30.03^{c} ± 3.29	18.68^{b} ± 2.64
	Stem	100.39^{d} ± 12.94	116.07^{d} ± 4.18	107.08^{d} ± 12.39
	Root	<LOD	<LOD	1.08^{a} ± 1.40
Epicatechin	Flower	6.14^{b} ± 1.24	$8.98^{c,d}$ ± 1.04	9.93^{d} ± 0.73
	Stem	176.94^{g} ± 2.13	171.65^{g} ± 6.76	64.54^{f} ± 5.45
	Root	8.12^{c} ± 0.46	16.11^{e} ± 0.59	3.29^{a} ± 0.16
Caffeic acid	Flower	2.52^{b} ± 0.49	2.87^{b} ± 0.70	9.30^{d} ± 1.13
	Stem	1.38^{a} ± 0.43	1.52^{a} ± 0.20	3.95^{c} ± 0.26
	Root	12.39^{e} ± 0.59	21.36^{f} ± 1.72	4.47^{c} ± 0.54
Vanillin	Flower	2.41^{a} ± 0.53	3.68^{b} ± 0.15	<LOD
	Stem	6.06^{c} ± 1.59	5.49^{c} ± 0.33	8.77^{d} ± 1.08
	Root	5.32^{c} ± 0.49	6.24^{c} ± 0.78	<LOD
***p*-Coumaric acid**	Flower	<LOD	<LOD	<LOD
	Stem	6.53^{a} ± 1.57	10.13^{b} ± 3.25	18.11^{c} ± 3.11
	Root	<LOD	<LOD	<LOD
Ferulic acid	Flower	<LOD	<LOD	13.12 ± 1.55
	Stem	6.17^{b} ± 0.55	7.22^{b} ± 0.93	2.66^{a} ± 1.45
	Root	<LOD	<LOD	12.256^{c} ± 7.45
4-hydroxybenzoic acid	Flower	<LOD	<LOD	<LOD
	Stem	0.47^{a} ± 0.04	0.66^{b} ± 0.07	0.78^{c} ± 0.03
	Root	1.22^{e} ± 0.04	1.12^{d} ± 0.03	2.29^{f} ± 0.17
Rutin	Flower	<LOD	<LOD	<LOD
	Stem	4.09^{a} ± 0.05	5.25^{c} ± 0.09	4.49^{a} ± 0.35
	Root	<LOD	<LOD	<LOD
Naringenin	Flower	0.17^{a} ± 0.03	0.15^{a} ± 0.06	<LOD
	Stem	0.31^{b} ± 0.03	0.26^{b} ± 0.03	<LOD
	Root	$0.33^{b,c}$ ± 0.07	0.34^{c} ± 0.05	0.17^{a} ± 0.02

LOD: Limit of detection

Free radical scavenging activity (DPPH●)

Antiradical activity is the amount of antioxidant required to reduce the concentration of DPPH by 50% and is indicated by IC50 (mg.mL-1). The lower the IC50 value calculated for total antioxidant activity, the higher the antioxidant activity. When the activity ranking was made among the solvents, the highest activity was observed in the stem part, while the lowest activity was observed in the flower part. When the same ranking was made for solvents, it was seen that the best activity was in methanol and the least activity was in ethanol. The best activity for all solvents and plant parts was found in the methanol extract of the stem part with an IC50 value of 0.15 mg.mL-1. The lowest activity was determined in the ethanol extract of the flower part with an IC50 value of 0.86 mg.mL-1. In this study, trolox was used as a standard and the IC50 value of trolox was 0.01 mg.mL-1. Compared to Trolox, methanol extracts of stem and root parts were found to have similar activity. These results are given in Figure 1c.

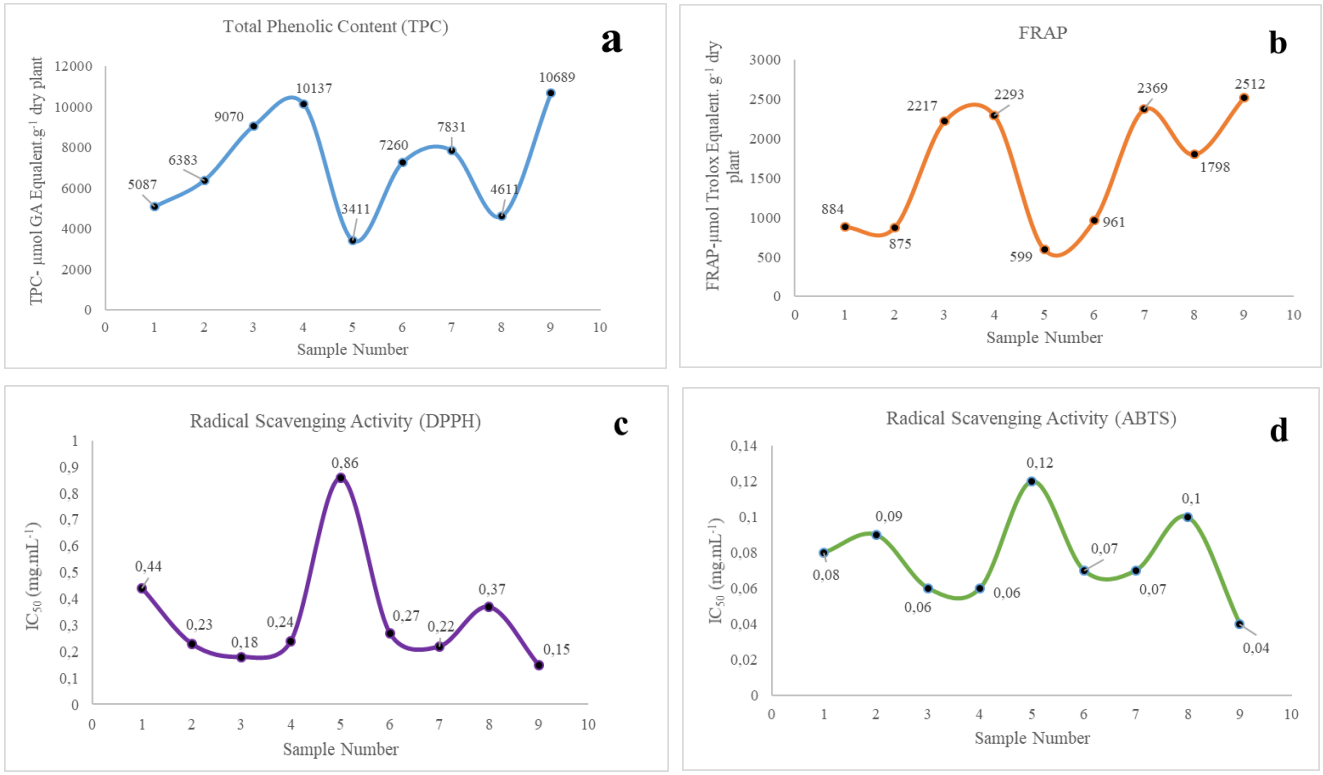
Cation radical scavenging activity (ABTS+)

As with DPPH, antiradical activity is the amount of antioxidant required to reduce the concentration of ABTS by 50% and is expressed as IC50 (mg.mL-1). The lower the IC50 value calculated for total antioxidant activity, the higher the antioxidant activity. The lowest activity was found in the ethanol extract of the flower part with an IC50 value of 0.12 mg.mL-1. When all solvents and plant parts were evaluated; the best activity was determined in the methanol extract of the stem part with an IC50 value of 0.04 mg.mL-1. Trolox was used as standard and the IC50 value of trolox was 0.004 mg.mL-1. These results are given in Figure 1d.

Determination of phenolic substance components of plants by LC-MS/MS

As seen in the Table 2. as a result of the analysis carried out with LC-MS/MS, the phenolic compounds and their amounts detected in different solvents and different parts of the plant are different.

The phenolic compounds detected in the highest amounts for all parts of the plant and solvents were catechin, epicatechin, chlorogenic acid, caffeic acid and vannilin. In the analysis results made with LC-MS/MS and Total phenolic substance test results, the highest amount of phenolic compounds and substances in both analyzes are found in the methanolic extract of the stem part of the plant. When the results of the antioxidant tests were evaluated collectively, the highest activity was observed in the methanol extract of the plant's stem. It was determined that the results obtained from the tests were consistent with each other.



Sample number and sample names: 1-Root-Water, 2-Root-EtOH, 3-Root-MetOH, 4-Flower-Water, 5-Flower-EtOH, 6-Flower-MetOH, 7-Stem-Water, 8-Stem-EtOH, 9-Stem-MetOH

Figure 1. a) Total Phenolic Content (TPC) graph, b) FRAP graph, c) Radical Scavenging Activity graph (DPPH) and d) Radical Scavenging Activity graph (ABTS)

Şekil 1. a) Toplam Fenolik İçerik (TPC) grafiği, b) FRAP grafiği, c) Radikal Temizleme Aktivitesi grafiği (DPPH) ve d) Radikal Temizleme Aktivitesi grafiği (ABTS)

In the FRAP and Total Phenolic Content (TPC) graphs, the highest values are the best examples in terms of activity. In contrast, for the Radical Scavenging Activity graph (ABTS and DPPH), the opposite is true. Sample number 5, that is, the ethanolic extract of the flower part, has the lowest activity.

Phenolic compounds were determined with LC-MS/MS. Twenty nine phenolic compounds were screened. Catechin, epicatechin, caffeic acid, chlorogenic acid, vanillin and ferulic acid were the phenolic compounds detected in the highest amounts in LC-MS/MS. In the LC-MS/MS study, p-coumaric Acid was found only in the stem part. It was observed that gallic acid and protocatechuic aldehyde was more concentrated in the root part.

In a study conducted by Başgedik et al. (2014), the antioxidant activity of Iris germanica, a species of Iris, was investigated. The researchers reported that I. germanica exhibits high antioxidant activity, which was attributed to the presence of various phenolic compounds. Analysis of the ethanol extract revealed the presence of protocatechuic acid, catechin, chlorogenic acid, p-hydroxybenzoic acid, caffeic acid, and ferulic acid. Based on these findings, the authors concluded that the antioxidant and antimutagenic properties of I. germanica extracts may contribute to the prevention of diseases such as heart disease, stroke, arteriosclerosis, and cancer (Basgedik et al., 2014).

A study with five different Iris species suggests that phenolic acids may be a group of pharmacologically active substances that could be responsible for the activity and applications of plants belonging to the Iridaceae family. This finding is significant because many phenolic acids, such as ferulic, caffeic and vanillic acids, not only have widely known antioxidant activity, but are also characterized by multiple pharmacological properties, including anti-inflammatory, antibacterial, and antimutagenic effects (Machalska et al., 2008).

The phenolic compounds found in the structure of Iris species are thought to explain their ethnomedicinal use in various places around the world. It has been stated that their high antioxidant activity constitutes a potential source with inflammatory, neuroprotective and hepatoprotective effects (Khatib et al., 2022).

In studies conducted with water and methanol extracts of another Iris species, I. persica subsp. persica, a median of 8 phenolic compounds were detected in both extracts. These phenolic compounds are shikimic acid, gallic acid, protocatechuic acid, vanillic acid, caffeic acid, o-coumaric acid, trans ferulic acid, sinapic acid and luteolin (Unver et al., 2024).

Caffeic acid, detected both in this study and in previous research on certain Iris species, is a notable phenolic compound. It has shown significant potential in cancer treatment and is recognized as a powerful natural antioxidant. Studies have demonstrated that caffeic acid induces apoptosis in cancer cells by increasing reactive oxygen species (ROS) levels and disrupting mitochondrial function. Additionally, it helps suppress metastasis, thereby reducing tumor aggressiveness. Its anti-inflammatory and antioxidant effects have also been confirmed in the context of 6-propyl-thiouracil (PTU)-induced hypothyroidism (Sun & Shahrajabian, 2023).

Another key phenolic compound identified is ferulic acid (4-hydroxy-3-methoxy cinnamic acid), a polyphenol widely known for its therapeutic benefits. It exhibits anti-aging, neuroprotective, and anti-inflammatory properties. Ferulic acid exists in both cis and trans isomeric forms, with the trans isomer being the most abundant in nature. Both isomers have been linked to beneficial effects in conditions such as diabetes, cancer, and neurodegenerative and cardiovascular diseases. In plants, ferulic acid contributes to cell wall rigidity. Furthermore, studies have indicated that ferulic acid, along with resveratrol, exerts antioxidant and antidiabetic effects by alleviating liver, kidney, and pancreas damage caused by alloxan-induced diabetes—likely through inhibition of the proinflammatory factor NF-κB. It also helps prevent radiation-induced lipid peroxidation and DNA damage while restoring antioxidant status and improving histopathological outcomes in experimental animals (Sun & Shahrajabian, 2023).

Two other important phenolic compounds that were identified were chlorogenic acid and quercetin. The same study found that chlorogenic acid has antidiabetic and anti-inflammatory properties, while quercetin has neuroprotective and antihypertensive effects (Mutha et al., 2021).

In this study, determination of phenolic substance components of plants by LC-MS/MS, total phenolic substance content, FRAP, ABTS and DPPH analyses of I. kerneriana plant grown in Çorum province and its villages in Turkey were performed. As a result of the findings, it was revealed that the plant has a high antioxidant capacity. ABTS and DPPH radical scavenging activity results are compatible with each other. In addition, the results of the collected phenolic substance content and FRAP analyses support each other. When the results of these four antioxidant activity tests were compared, it was found that the methanol extract of the root exhibited the highest activity. As a result of literature research, it was stated that other Iris species have high antioxidant activity, this study revealed that the antioxidant activity of I. kerneriana, an Iris species, was high.

It is also compatible with the phenolic compounds determined in studies conducted on different Iris species in the literature. It is thought that the plant has high antioxidant activity due to the phenolic compounds determined. In addition, it is known that the phenolic compounds determined have heart disease, stroke, arteriosclerosis, cancer, anti-inflammatory, antibacterial and antimutagenic effects.

ACKNOWLEDGEMENTS

This study was financially supported by TUBITAK (2209-A, Project No; 1919B012318822). The authors thank for the financial support. In addition, this study has been supported by Anadolu University Scientific Research Projects Coordination Unit under grant number 2206S046.

STATEMENT OF CONFLICT OF INTEREST

The authors declare that there is no conflict of interest between them.

AUTHOR'S CONTRIBUTIONS

Conception and design of the study: Hacer Doğan., Ömer Kayır, Bahar Cömert.; sample collection: Ömer Koray Yaylacı; analysis and interpretation of data: Hacer Doğan, Ömer Kayır, Bahar Cömert; statistical analysis: Hacer Doğan, Ömer Kayır; visualization: Hacer Doğan, Ömer Kayır; writing manuscript: Hacer Doğan, Ömer Kayır.

STATEMENT OF ETHICS CONSENT

This article does not require ethical approval as there are no experiments with human or animal subjects.

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