



Phenolic profile, fatty acid and mineral composition with antioxidant, antibacterial, and enzyme inhibitor activities of different extracts from *Erodium Cicutarium* (L.) L'Hér. consumed as a vegetable in Kilis, Turkey

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Abstract

This study aimed to explore the antioxidant activities, phenolic profile, fatty acid and mineral composition of the different extracts of *Erodium cicutarium* (L.) L'Hér. consumed as a vegetable in Kilis, Turkey. While catechine hydrate was the most abundant phenolic compounds in methanol, ethanol and diethyl ether extracts, it was chlorogenic acid in the water extract. Looking at the fatty acid profile, the amount of palmitic acid, one of the saturated fatty acids, was found to be high (34.30%), followed by stearic acid (5.10%). Total monounsaturated fatty acids are the second highest fatty acids. Total polyunsaturated fatty acids were determined as the third rank fatty acids. While the amount of linoleic acid, one of the total polyunsaturated fatty acids, was determined as 17.62%, the ratio of linolenic acid was determined as 9.60%. While the most calcium, magnesium and potassium were found among the 9 different mineral substances determined, respectively; the lowest element was found to be nitrogen. Looking at the results, it was determined that the plant is a high source of calcium (1078.503 mg/kg). Inhibitory effects against *Pseudomonas aeruginosa* ATCC 9027 were observed, with a zone diameter of 5.5 mm and 11 mm in methanol extract and diethyl ether extract, respectively. Ethanol and water extracts of *E. cicutarium* (L.) L'Hér. may be preferred as an alternative natural agent due to their amylase and tyrosinase activities. The results suggested that the *E. cicutarium* (L.) L'Hér. extracts may be useful for food and medicinal applications.

Keywords Antioxidant · *Erodium cicutarium* · Fatty acid · Mineral · Phenolic compounds

Introduction

In Turkey, the taxa that make up the genus *Erodium* L'Her. and are members of the family Geraniaceae (Turnagagasigiller) and are referred to as “dönbaba” and “iğnelik.” The *Erodium* genus, consisting of 26 species in our flora, is documented for its diverse ethnobotanical applications in addressing conditions such as diabetes, edema, constipation, tooth and stomach pain, liver disorders, and inflammation. Some *Erodium* species are also eaten as vegetables in some nations, including Turkey, the North American region, and Italy [1]. *E.cedrorum*, *E.cicutarium* and *E.laciniatum*, which are species belonging to the *Erodium* genus, comprised of 26 species and 31 taxa in the flora of Turkey are consumed

as vegetables in some provinces (Afyonkarahisar, Ankara, Aksaray and Denizli). Moreover, *E. cicutarium* stands out as the most used species for ethnobotanical purposes [1].

It originates from the Mediterranean region and features fern-like, pinnate leaves measuring 10–30 cm in length. These leaves emerge from a rosette and possess a “green” grassy flavor. It has little, five-petalled pink flowers that are annual or biennial. The fruits, which are made up of five mericarps linked together, grow in a manner resembling a huge spine after flowering. Due to the widespread usage of *Erodium* species in traditional medicine, there is a wealth of ethnopharmacological information available in the literature. As a traditional medicine, *E. cicutarium* has been applied topically and internally to treat worm infections, fever, wounds, and dysentery. It was used as a general haemostipticum and as an antihemorrhagic medication in gynecology to control uterine hemorrhage. It was one of several plants in the *Erodium* genera that contained ellagitannins and was used to make antibacterial and astringent teas

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for stomatitis. Additional ethnopharmacological evidence points to its use as an abortifacient, as well as for nephritis, hepatitis, influenza, stomach ache, heart issues, bleeding from wounds, rashes and ulcers [2].

In the study conducted by Nikolova et al. [3], it was observed that the free radical scavenging effect of *E. cicutarium* extract surpassed that of the four Geranium species examined.

One important class of chemicals thought to contribute to the antioxidant action of fruit and vegetable by-products are phenolic compounds [4]. The most prevalent secondary metabolites are polyphenolic chemicals, which are gaining popularity because of their potential uses in a variety of chemical technology domains [5]. According to Bilic et al. [6], literature data and their previous research indicate a substantial polyphenolic composition, including hydroxycinnamic acids, flavonoids, and tannins. Additionally, the presence of fatty acids, saponins, amino acids, sugars, and a chemically diverse essential oil was noted, with hexadecanoic acid standing out as a prominent compound. Researchers reported that data from the literature and our earlier research demonstrate the great variety of phytochemical components found in *E. cicutarium* extracts, which served as the inspiration for our bioactivity investigation.

Depending on the mechanism of the relevant chemical reactions, antioxidant capacity analyzes are divided into 3 classes as: (i) single electron transfer (SET), (ii) hydrogen atom transfer (HAT) reaction-based assays, or (iii) chelation of transition metals. While SET-based assays measure the reducing capacity of the antioxidant, HAT-based assays measure its capacity to donate hydrogen atoms. The purpose of HAT and SET-based assays is to measure the radical scavenging capacity of a sample rather than its preventive antioxidant capacity. The third type of mechanism of action of AOs is their ability to chelate transition metals, namely Zn^{2+} , Fe^{2+} , and Cu^{2+} . Chelation of transition metals can also be used to estimate the antioxidant capacity of an extract [7]. Inhibition of induced low-density lipoprotein autoxidation, oxygen radical absorbance capacity (ORAC), total radical trapping antioxidant parameter (TRAP) and crocin bleaching assay are HAT-based, while Folin–Ciocalteu reagent (FCR), Trolox equivalence antioxidant capacity (TEAC) analyzes are SET-based [8].

Since different antioxidant compounds may have different effects within the cell, no single method can fully reflect the antioxidant content of foods, so it is more appropriate to use more than one technique to evaluate the entire antioxidant spectrum available [9].

The aim of this study, the phytochemical contents (ascorbic acid, total flavonoid content, total phenolic content), antioxidant (chelating iron ions, phosphomolybdenum, CUPRAC, FRAP, ABTS radical cation removal, DPPH

radical removal test) and antibacterial activities of water, ethanol, methanol, diethyl ether extracts obtained from *E. cicutarium* (L.) L'Hér. and to determine phenolic profile, the fatty acid composition and mineral components of the plant.

Materials and methods

Preparation of different extracts from *Erodium cicutarium* (L.) L'Hér.

The plant material (*E. cicutarium* (L.) L'Hér.) to be used in this study was obtained from the Kilis Provincial Directorate of Agriculture and Forestry in Kilis (Location information: 2169 Island, 6 parcels, O38-D-03-D-3-A map, 46,730.39 title deed area, Kilis, Merkez, Ebulüle Mah). Dr. Olcay Ceylan identified the species and placed it in the Herbarium of the University of Mugla Sitki Kocman in Turkey (Voucher Number: O.1343). After the herbal material was dried in a cool environment without direct sunlight, it was ground in a coffee and spice grinder (Arçelik K 3104) and then ground into powder. The powdered plant underwent extraction using water, methanol, ethanol, and diethyl ether solvents at a ratio of 1:10 (w/v) with agitation at room temperature for 72 h. Once the extraction process was concluded, the samples were filtered through Whatman filter paper No.1. The resulting extracts were evaporated using an evaporator based on the boiling temperature of each solvent. Following evaporation, the samples were reconstituted in methanol at a final concentration of 100 mg/mL and stored at +4 °C for subsequent analysis. The extracts obtained in the analyzes were diluted and the analyzes were continued at 10 mg/mL for each solvent.

Phytochemical analyzes

Total phenolics, total flavonoids, ascorbic acid amount and DPPH analyzes in the different extracts of *E. cicutarium* (L.) L'Hér. were performed in our previous study, as outlined by Ucan Türkmen and Mercimek Takci [10].

Determinatin of IC_{50} value

0.1 mM DPPH prepared in methanol was added to 1 mL of extract at different concentrations (100–1000 $\mu\text{g/mL}$). After incubation at room temperature and in the dark for 30 min, the residual amount of DPPH was mesasured at 517 nm. Control was also performed utilizing 1 mL of methanol instead of the sample. Inhibition of DPPH was calculated as percent by following formula.

$$\text{Inhibition (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100.$$

50% inhibition value of DPPH are defined as IC_{50} value. This value was calculated by using dose-response curve plotting between % inhibition and concentrations and the results were given as $IC_{50} = \mu\text{g/mL}$ [10].

Antioxidant analyzes

Metal chelating activity

3.7 mL of deionized water and 100 μL of 2 mM FeCl_2 answer have been delivered to at least 1 mL sample. Then incubated at room temperature for 30 min, 200 μL of 5 mM ferrozine answer becomes delivered and mixed. After 10 min, the absorbance of the aggregate becomes measured at 562 nm. Control becomes additionally done by the usage of 1 mL of deionized water in preference to the sample. As standard, EDTA answers at 50–250 $\mu\text{g/mL}$ concentrations have been used. According to the following equation, the steel chelating interest of extracts becomes calculated [11].

$$\text{Metal chelating activity (\%)} = [(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100.$$

Reducing power assay (FRAP)

100 mg/mL concentrations and standard solutions (20–400 $\mu\text{g/mL}$) (1 mL) of extract with 2.5 mL 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL solution. 1% potassium ferricyanide, incubate the mixture at 50 °C for 20 min, add 2.5 mL of 10% trichloroacetic acid (w/v), and centrifuge the mixture at 2500 rpm for 10 min. The upper layer (5 mL) is mixed with 5 mL of distilled water and 1 mL of 0.1% ferric chloride, and the absorbance is measured at 700 nm. The test is performed in triplicate. The results are expressed as the average $\pm$ standard value. The deviations of BHA, BHT, ascorbic acid, and α -tocopherol are used as standard [11].

Trolox equivalent antioxidant capacity assay (TEAC)

A solution was prepared by combining 7 mM ABTS with 2.45 mM potassium persulfate. The resulting solution was incubated at 20 °C for 12 and 16 h to generate the ABTS radical. PBS (phosphate buffer; Phosphate Buffer Saline) solution, used for diluting the radical solution, samples, and Trolox standard, is prepared. 8.77 g of NaCl was added to 0.1 M phosphate buffer. It was adjusted to a pH of 7.4. Before starting the analysis, 1 mL of ABTS radical solution was taken and diluted with approximately 90–100 mL of PBS to an absorbance of 0.700 ± 0.02 at 734 nm. After, extract and PBS were

mixed. The absorbance was measured per minute for 6 min. The findings were presented in terms of TEAC (Trolox Equivalent Antioxidant Capacity) values, as indicated by Ucan Türkmen et al. [12].

Phosphomolybdenum method

Spectrophotometric analysis employing the phosphomolybdenum method was utilized to determine the total antioxidant capacity, as outlined by Zengin et al. [13]. In this method, 3 milliliters of a reactive solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) was swiftly mixed with 300 μL of the extract. Following an incubation period at 95 °C for 90 min, the absorbance was measured at 695 nm using a Biochrom Libra S60 spectrophotometer. The total antioxidant capacity was expressed in trolox equivalents ($\mu\text{g/TE g}$).

CUPRAC

For CUPRAC (cupric ion reducing activity), a sample solution (0.5 mL) was introduced into a pre-mixed reaction mixture consisting of CuCl_2 (1 mL, 10 mM), neocuproine (1 mL, 7.5 mM), and NH_4AC buffer (1 mL, 1 M, pH 7.0). Likewise, a blank was created by combining 0.5 mL of the sample solution with a pre-mixed reaction mixture (3 mL) that lacked CuCl_2 . Similarly, a blank was prepared by adding a sample solution (0.5 mL) to a pre-mixed reaction mixture (3 mL) without CuCl_2 . Subsequently, the absorbance of the sample and blank was measured at 450 nm after a 30-minute incubation at room temperature, following the procedure described by Baltaci et al. [14].

Determination of phenolic compounds

The extracts underwent filtration through a 0.45 μm teflon membrane filter before being injected into the HPLC instrument. High-performance liquid chromatography (HPLC) analyses were conducted using an Agilent 1260 infinity series system, which included a quaternary pump, a column temperature control oven, an autosampler unit, and a detector (DAD) 0.10 μL of supernatant was injected into the C18 (Ace Generix; 4.6 mmx250) column. The column was kept at 30 °C and the detector was set at 200 and 300 nm. The mobile phases (83% fosforic acid (A), 17% acetonitrile (C)) - flowed as gradient at flow rate of 0.8 mL/min. The gradient elution was 83% A + 17% C at 0 min, 85% A + 15% C at 7 min; 80% A + 20% C at 20 min, 75% A + 25% C at 24 min, 70% A + 30% C at 28 min, 60% A + 40% C at 30 min, 50% A + 50% C at 32 min, 30% A + 70% C at 36 min and 83% A + 17% C at 40 min [15, 16].

Determination of fatty acid composition

The fatty acids of the extracted oils were first esterified and then analyzed by GC-MS (Shimadzu Nexis GC-2030, Kyoto, Japan). To obtain fatty acid methyl esters, 20–60 mg of oil sample was weighed into a tube and 2 mL of hexane was added. Then, after adding 0.2 mL of 2 M methanolic potassium hydroxide solution, the contents of the tube were mixed in vortex for 2 min. After phase separation, the supernatant was taken and filtered and transferred to the vial [17]. After the fatty acid methyl esters were prepared, the fatty acid profile was determined as % by GC-FID.

Mineral substance content

For this purpose, 2 g of sample was taken, transferred to teflon tubes and burned in a microwave oven with 2 mL of 65% nitric acid (HNO_3) and 1 mL of 30% hydrogen peroxide (H_2O_2) added. The solutions obtained as a result of the combustion process were transferred to a 25 mL balloon jug with the help of a funnel and completed with ultrapure water up to the volume of the balloon. The dilution required for the determination of each mineral substance was made over the first dilution process [18]. After the preliminary preparations were made for the determination of the mineral substance, the mineral amounts were calculated in mg/kg by atomic absorption spectrometry.

Antibacterial activity

The antibacterial activity of the extracts was investigated using the Kirby–Bauer disk diffusion susceptibility test, as described by Bauer et al. [19]. For this particular method, a total of 3 bacteria species (*Escherichia coli* ATCC 25922, *Proteus spp.*, *Pseudomonas aeruginosa* ATCC 9027) were used for the antimicrobial test. Cultures in growth Luria–Bertani (LB) broth were incubated for 24 h, and the density was adjusted to 0.5 MacFarland turbidity. The antibacterial activity was assessed on Mueller Hinton Agar. The sterile antimicrobial blank discs, each impregnated with 20 μL of the extracts, were strategically positioned with adequate separation. Negative and positive controls utilized methanol solvent and standard antibiotics, respectively. Tetracycline for *E. coli* ATCC 25,922 and *Proteus spp.*; Polymyxine for *P. aeruginosa* ATCC 9027 was used as the positive control. After incubating at 37 °C for 24 h, the areas devoid of bacterial growth surrounding the discs were assessed as indicators of antibacterial activity.

Enzyme inhibitory activity

Inhibitory effects of the extracts of *E. cicutarium* (L.) L'Hér. on tyrosinase and α -amylase were carried out accordingly Sarikurkcü et al. [20]. The measurement of tyrosinase inhibitory activity involved a modified dopachrome method with L-DOPA as the substrate. Inhibitory activity on α -amylase was assessed using the Caraway Somogyi iodine/potassium iodide (IKI) method.

Statistical analysis

The outcomes from the water, ethanol, methanol, and diethyl ether extracts of *E. cicutarium* (L.) L'Hér. were analyzed for variance using the SPSS 23.0 software package. Significant differences were identified based on Duncan's multiple comparison test, with a confidence level of $p=0.05$.

Results and discussion

Phytochemical contents and antioxidant activities of different extracts of *Erodium cicutarium* (L.) L'Hér.

The percentage extraction yield of the *E. cicutarium* (L.) L'Hér. were calculated as 12.84%, 14.29%, 4.49% and 5.17% in the methanol, water, ethanol and diethyl ether extracts, respectively. The high yield was observed in aqueous extracts. However, the yield of the diethyl ether extracts was lower than that of the other extracts. The polarity rate of the employed solvent is a significant element that is crucial for the compound's solubility in plant samples [21–23].

As seen in the Table 1, phytochemical contents of different *E. cicutarium* (L.) L'Hér. extracts were determined by examining the amounts of total phenolic substance (TPC), total flavonoid substance (TFC) and ascorbic acid amount (AAA) ($p<0.05$). TPC of the different extracts *E. cicutarium* (L.) L'Hér. ranged from 0.412 ± 0.02 to 0.605 ± 0.00 to mg GAE/g. A high TPC value was detected in the methanol extract. While the highest TPC value was detected in the methanol extract, the lowest TPC value was in the diethyl ether extract. The highest TFC was identified in methanol extract, 0.036 ± 0.01 mg RE/g. The TFC was noted as minimum in diethyl ether extracts (0.015 ± 0.00 mg RE/g). AAA of the extracts ranged from 51.66 ± 0.29 mg/L to 110.57 ± 14.50 mg/L. As with the TPC value, while the highest AAA value was determined the ethanol extract, the lowest AAA value was in the water extract (Table 1).

As seen in the Table 1, antioxidant activities of different *E. cicutarium* (L.) L'Hér. extracts were determined by examining the DPPH removal activities, phosphomolybdenum, chelating activities, reduction power, TEACs and CUPRAC

Table 1 The phytochemical contents and antioxidant activities of water, ethanol, methanol and diethyleter extracts of *Erodium cicutarium* (L.) L'Hér.

Extracts (10 mg/mL)	Total Phenolic Content (TPC) (mg GAE/g)	Total Flavonoid Content (TFC) (mg RE/g)	Ascorbic Acid Amount (AAA) (mg/L)	DPPH Removal Activities of Extracts (DPPH: Inhibition) (%)	Phosphomolybdenum (TE g)	Chelating Iron Ions (%)	FRAP (abs.)	CUPRAC (mg trolox equivalent/g sample)	TEAC (Inhibition %)	TEAC (μM trolox equivalent/10 g)
E _{Water}	0.443 ± 0.01 ^b	0.018 ± 0.00 ^b	51.66 ± 0.29 ^b	74.00 ± 8.66 ^a	0.050 ± 0.00 ^a	7.52 ± 0.18 ^d	0.142 ± 0.01 ^d	67.43 ± 6.03 ^a	74.60 ± 0.20 ^a	19.88 ± 0.00 ^a
E _{Ethanol}	0.594 ± 0.00 ^a	0.020 ± 0.00 ^b	110.57 ± 14.50 ^a	65.34 ± 1.88 ^b	0.050 ± 0.00 ^a	14.64 ± 0.18 ^c	2.282 ± 0.04 ^a	77.42 ± 4.23 ^a	41.09 ± 0.29 ^c	10.79 ± 0.06 ^c
E _{Methanol}	0.605 ± 0.00 ^a	0.036 ± 0.01 ^a	52.17 ± 0.29 ^b	77.13 ± 1.74 ^a	0.049 ± 0.00 ^a	27.86 ± 0.27 ^b	1.962 ± 0.13 ^b	78.22 ± 9.31 ^a	59.14 ± 1.79 ^b	15.78 ± 0.44 ^b
E _{Diethyl ether}	0.412 ± 0.02 ^c	0.015 ± 0.00 ^b	72.82 ± 19.57 ^b	19.64 ± 0.81 ^c	0.049 ± 0.00 ^a	77.29 ± 0.27 ^a	0.387 ± 0.02 ^c	17.20 ± 0.72 ^b	13.52 ± 2.12 ^d	2.22 ± 0.21 ^d

*(The data shown are the mean values of $n = 3$. The difference between the values expressed with different symbols (a-c; a-b; a-d) in the same columns in the graph was significant ($p < 0.05$))

Table 2 IC₅₀ values of extracts (μg/mL)

Extracts	IC ₅₀
E _{Water}	3.36
E _{Ethanol}	2.72
E _{Methanol}	2.40
E _{Diethyl ether}	11.03

($p < 0.05$). DPPH removal activities of extracts ranged from $19.64 \pm 0.81\%$ (diethyl ether extract) to $77.13 \pm 1.74\%$ (methanol extract) (Table 1).

The antioxidant concentration removed 50% of the DPPH radical is defined as the IC₅₀ value. The low IC₅₀ value is indicated high radical removal activity. These values are shown in Table 2.

As seen in Table 2, the lowest IC₅₀ dose was reported for methanol extract (2.40 μg/mL). According to IC₅₀ values, free radical removal activity of methanol extract was dramatically higher than that of the other extracts. The diethyl ether extract having the highest IC₅₀ value showed the lowest free radical scavenging activity.

As shown in the Table 1, phosphomolybdenum assay antioxidant values of the different extracts ranged from 0.049 ± 0.00 (in methanol and diethyl ether extracts) to 0.050 ± 0.00 μg TE/g (in water and ethanol extracts). As demonstrated by in Table 1, the chelating activities of the different extracts at 10 mg/mL concentration were detected as 7.52% and 77.29% in the water and the diethyl ether extracts, respectively. Chelating activity was assessed in comparison to EDTA, a common chelator. Its activity was less than EDTA's (80.16–94.42%) activity (Table 3). Also, Table 1 clearly demonstrates that the reduction power of extracts ranged from 0.142 ± 0.01 to 2.282 ± 0.04 at 10 mg/mL concentration values. The outcomes were contrasted with standards for α-tocopherol, BHT, ascorbic acid and BHA ($p < 0.05$) (Table 3). TEACs of the extracts were determined as 2.22 ± 0.21 and 19.88 ± 0.00 μM Trolox equivalent/10 g in the diethyl ether extract and the water extract, respectively. Similar to this, the TEACs of the extracts were computed as inhibition%, with values for the diethyl ether extract and the water extract coming out to 13.52% and 74.60%, respectively (Table 1). At a concentration of 10 mg/mL, the CUPRAC of the extracts ranged from 17.20 to 78.22 mg trolox equivalent/g sample (Table 1). The methanol extract had the highest CUPRAC value, whereas the diethyl ether extract had the lowest CUPRAC value. The outcomes were contrasted with the trolox standard ($p < 0.05$) (Table 3). When all phytochemical and antioxidant results are taken into consideration, the highest results in TPC, TFC, DPPH and CUPRAC analyzes were found in methanol extracts. While ethanol extracts gave the highest results in AAA and FRAP analysis, water extract gave the highest results in TEAC analysis.

Table 3 Used standards for FRAP, Chelating Iron Ions (Ucan Turkmen et al., 2020) and CUPRAC

Analys	Standards	Concentrations				
		20 µg/mL	50 µg/mL	100 µg/mL	200 µg/mL	400 µg/mL
FRAP	BHT	0.07 ± 0.00 ^c	0.09 ± 0.00 ^d	0.14 ± 0.00 ^c	0.33 ± 0.02 ^b	0.45 ± 0.01 ^a
	BHA	0.22 ± 0.01 ^d	0.24 ± 0.01 ^{cd}	0.25 ± 0.02 ^c	0.32 ± 0.03 ^b	0.51 ± 0.01 ^a
	α-tocopherol	0.13 ± 0.00 ^c	0.15 ± 0.00 ^d	0.16 ± 0.01 ^c	0.20 ± 0.01 ^b	0.29 ± 0.01 ^a
	Ascorbic acid	0.12 ± 0.00 ^c	0.13 ± 0.00 ^d	0.17 ± 0.00 ^c	0.25 ± 0.00 ^b	0.37 ± 0.00 ^a
Chelating Iron Ions	EDTA	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
		80.16 ± 1.34 ^b	93.59 ± 0.83 ^a	94.42 ± 0.00 ^a	94.83 ± 0.00 ^a	94.73 ± 0.10 ^a
CUPRAC	Trolox	50 µg/mL	100 µg/mL	150 µg/mL	200 µg/mL	250 µg/mL
		5.25 ± 0.01 ^c	11.33 ± 0.27 ^d	17.17 ± 0.42 ^c	21.34 ± 0.61 ^b	26.62 ± 0.42 ^a

*(The data shown are the mean values of $n=3$. The difference between the values expressed with different symbols (a-e; a-d; a-b) in the same lines in the graph was significant ($p<0.05$))

Celikler [24] found the radical scavenging effect of DPPH as 91.97% in the ethanol extract of *E. cicutarium* at a stock concentration of 2000 µg/mL in his doctoral thesis study. The researcher determined the percent yield of ethanol extract at the same concentration as 2.89%. This value was found lower than our ethanol extract. Moreover, the researcher determined the FRAP value as 1.045 (abs.).

According to the correlation results between CUPRAC, FRAP, DPPH and ABTS; There is no significant relationship between DPPH and FRAP ($r=0.419$; $p>0.01$). There is a significant relationship between DPPH and CUPRAC ($r=0.935$; $p<0.01$). There is no significant relationship between FRAP and CUPRAC ($r=0.459$; $p>0.01$). There is a significant relationship between CUPRAC and ABTS ($r=0.798$; $p<0.01$). There is a significant relationship between ABTS and DPPH ($r=0.896$; $p<0.01$). There is no significant relationship between ABTS and FRAP ($r=0.009$; $p>0.01$).

The antioxidant activity of methanolic extracts prepared from the aerial parts of 9 plant species (8 *Geranium* sp. and 1 *Erodium* sp.) belonging to the Geraniaceae family, collected from various regions of Bulgaria, was investigated by DPPH radical scavenging method. All extracts showed strong antioxidant effects, and the free radical scavenging effect of *E. cicutarium* extract was found to be higher than the 4 *Geranium* species screened in the same study [3, 24].

Ten of the *Erodium* taxa screened showed a higher (>90%) DPPH radical scavenging effect than the reference compound quercetin (88.85%), and *E. birandianum* (91.11%) had the highest activity. In the FRAP experiment, 13 *Erodium* extracts were more active than the reference compound quercetin, and the highest FRAP activity was found in the *E. somanum* extract [24].

As reported in many studies, the high antioxidant activities of plants and their rich polyphenol content are generally directly proportional [24–28].

Bilic et al. [29] explored the antioxidant activity of water and methanolic extracts of *E. cicutarium* from four locations in Croatia (Buzin, Trešnjevka, Plitvice, Podvinje).

The CUPRAC analysis revealed that the antioxidant activities of methanolic extracts ranged from 36.13 to 78.34 mg trolox equivalent/g sample, while water extracts exhibited values between 17.19 and 46.85 mg trolox equivalent/g sample. This result in methanolic extract (78.34 mg trolox equivalent/g sample) is similar to our study (78.22 mg trolox equivalent/g sample).

Barba et al. [30] reported that hydroethanolic extracts of *Erodium* had significantly higher antioxidant activity than aqueous extracts, as measured by the Trolox equivalent antioxidant capacity (TEAC; 21.17 mM TE) (3.04 mM TE). A higher TEAC score indicates increased activity of our extracts, as the TEAC value is recognized for assessing antioxidant activity. Similarly, in our study, the TEAC value of water extracts was found to be higher when compared to other extracts.

Kayani et al. [31] explored the distribution of secondary metabolites in plants in the Quetta-Balochistan region. They reported that of total phenolic contents of *E. cicutarium* were detected as 0.18 mg/g. Moreover, these researchers were found to alkaloids in *E. cicutarium*. They noted that the six plants which are free of alkaloids, saponins and tannins, contain considerable quantity of total phenolics. They mentioned that the increased total phenolics level can be ascribed to the presence of phenolic substances such as lignans, coumarins, flavonoids, lignans, neolignans, lignins, phenylpropenes in the afore said plants.

Sroka et al. [32] identified that extracts from *E. cicutarium* contained catechins, tannin, sugars (fructose, galactose, glucose), elagic and gallic acids, amino acids (glutamic acid, alanine, glycine, tryptophan, histidine, proline, tyrosine), and vitamins C and K. They found that only polyphenolic substances, such as vitamin C, tannin, gallic acid, and (+)-catechin, displayed potent antioxidative effects. These researchers said that polyphenolic substances and vitamin C are present in the water and ethyl acetate extracts that hydrophilic fractions.

Sarikurkcü et al. [33] reported that yield obtained from the ethanol extracts of *E. cicutarium* was 3.05%.

Moreover, they calculated- 2.04 mmol TEs/g extract antioxidant activity for phosphomolybdenum assay in the *E. cicutarium* ethanol extract. Additionally, DPPH free radical scavenging activity, chelating effect, scavenging ability of *E. cicutarium* in ABTS cation radical scavenging assay and CUPRAC were found to 90.60 mg TEs/g extract, 22.22 mg EDTAEs/g extract, 129.12 mg TEs/g extract and 130.44 mg TEs/g extract, respectively. According to Sarikurkcu et al. [33], phenolic content and antioxidant activity of *E. cicutarium* show a substantial link. The overall flavonol concentrations and antioxidant capacities of the plant species were shown to be significantly correlated, according to the researchers.

The authors compared the water and methanolic extracts of *E. cicutarium* using the CUPRAC, DPPH, FRAP and ABTS assays. Among the four assays conducted, namely CUPRAC, ABTS and DPPH, three indicated that the methanolic extraction exhibited a higher antioxidant capacity than the water extract [34]. Our results are similar to the analyzes stated by the authors, except for ABTS.

Phenolic compounds of different extracts of *Erodium cicutarium* (L.) L'Hér.

Phenolic compounds of different extracts of *Erodium cicutarium* (L.) L'Hér. shown in the Table 4. The extraction in which the most phenolic compounds were obtained was the methanol extraction (totally 1048.2285 mg/kg). The methanol extraction was followed by water (891.678 mg/kg), ethanol (680.4766 mg/kg) and diethyl ether (140.3009 mg/kg) extractions respectively. According to this result, it may be possible to say that the best extraction for extracting phenolic compounds is methanol extraction. Our findings indicated that methanol extraction was adequate for protecting these components. Additionally, compounds such as phenolics and flavonoids demonstrate increased solubility in solvents with higher polarity, attributed to the high polarity of the HO- groups [35].

The total phenolic contents in the methanol, water, ethanol, and diethyl ether extracts varied between 2.09 and 797.53 mg/kg, 1.75 to 539.74 mg/kg, and 1.49 to 370.59 mg/kg, respectively. Among these extracts, catechine hydrate was the predominant phenolic compound in the methanol, ethanol, and diethyl ether extracts, while chlorogenic acid

Table 4 Phenolic compounds of the different extracts of *Erodium cicutarium* (L.) L'Hér.

Phenolic compounds	Extracts			
	Water	Methanol	Ethanol	Diethyl ether
Phenolic acids				
Hydroxybenzoic acids (mg/kg)				
Vanillic acid	n.d.	4.73	n.d.	n.d.
4-Hydroxy benzoic acid	1.99	3.75	n.d.	n.d.
Hydroxycinnamic acids (mg/kg)				
<i>o</i> -coumaric acid	2.68	7.32	12.81	2.53
<i>t</i> -Ferulic acid	1.75	n.d.	n.d.	n.d.
Caffeic acid	n.d.	16.37	12.25	1.11
<i>p</i> -coumaric acid	n.d.	2.09	1.49	n.d.
Chlorogenic acid	539.74	n.d.	n.d.	n.d.
3-Hydroxycinnamic acid	3.96	38.96	38.40	7.73
Rosmarinic acid	7.12	2.45	2.34	n.d.
Salicylic acid	2.46	58.22	51.75	n.d.
<i>t</i> -Cinamic acid	n.d.	n.d.	7.69	n.d.
Flavonoids				
Flavan-3-ols (mg/kg)				
Catechine hydrate	322.67	797.53	370.59	693.99
Flavanones (mg/kg)				
Naringine	n.d.	55.61	44.14	n.d.
Flavonols (mg/kg)				
Rutin	5.16	3.80	n.d.	n.d.
Quercetin	n.d.	n.d.	10.51	5.86
Flavones (mg/kg)				
Chrysin	9.83	36.44	123.07	53.13
Flavones	5.33	14.21	n.d.	n.d.
Stilbenoids (mg/kg)				
Resveratrol	n.d.	10.10	5.38	5.13

*n.d. not detected

Table 5 Fatty Acid Composition of *Erodium cicutarium* (L.) L'Hér.

Fatty Acid	Amount (%)
C16:0	34.30 ± 0.27
C18:0	5.10 ± 0.16
C18:1	31.06 ± 0.74
C18:2	17.62 ± 0.30
C18:3	9.60 ± 0.11
C20:1	2.38 ± 1.20
ΣSFA	39.40 ± 0.11
ΣMUFA	33.44 ± 0.46
ΣPUFA	27.22 ± 0.42

* C16:0:Palmitic acid, C18:0:Stearic acid, C18:1:Oleic acid, C18:2:Linoleic acid, C18:3:Linolenic acid, C20:1:Gadoleic acid, ΣSFA: Total saturated fatty acids, ΣMUFA: Total monounsaturated fatty acids, ΣPUFA: Total polyunsaturated fatty acids

dominated in the water extract. But, value of catechine hydrate in diethylether extract was higher than other extracts (693.99 mg/kg). This result contradicts many studies and literature that show that solvents with high polarity such as ethanol or methanol are better solvents to extract phenolic content than water.

Fecka and Cisowski [36] elucidated the structure of the principal phenolic components of *E. cicutarium*, including protocathechuic acid, 3-O-galloylshikimic acid, gallic acid, 3-O-(6''-O-galloyl)-β-D-galactopyranoside, didehydrogeraniin (dehydrogeraniin), corilagin, hyperin, geraniin, methyl gallate 3-O-β-D-glucopyranoside, isoquercitrin and rutin, through the combination of two analytical techniques: mass spectrometry and nuclear magnetic resonance [37].

In methanol extracts from *E. cicutarium*, the predominant phenolic acids and depsides were protocathechuic acid, gallic acid, brevifolin, gallic acid methyl ester, and ellagic acid. The respective quantities for each were 12.40, 3.93, 18.38, 25.95, and 11.88 mg per gram of dry weight [38, 39].

The presence of diverse hydroxycinnamic acid derivatives in *E. cicutarium*, which Bilic et al. [29] previously documented, including caffeic acid, ferulic and p-coumaric, followed by vanillic acid and p-hydroxybenzoic acid, support the findings in *E. hendrikii*. Rutin and narcissin were recently identified as the two main flavonoid components in *E. cicutarium* samples taken from four sites in Croatia by Bilic et al. [29]. The literature has given rutin, a well-known flavonoid, a lot of attention because of its wide range of pharmacological activities, which include antibacterial, antidiabetic, anticancer, anti-inflammatory, antithrombotic and antiallergic activity [29, 37, 40].

Fatty acid composition of *Erodium cicutarium* (L.) L'Hér.

The fatty acid composition of *Erodium cicutarium* (L.) L'Hér. was determined and the results are given in Table 5. Looking at the fatty acid profile, 60.6% of the fatty acid profile consisted of unsaturated fatty acids, 2 of which were monounsaturated and 2 were polyunsaturated. In particular, the amount of palmitic acid, one of the saturated fatty acids, was found to be high (34.30%), followed by stearic acid (5.10%). Total monounsaturated fatty acids are the second highest fatty acids. While the amount of oleic acid, one of the monounsaturated fatty acids, is 31.06%, gadoleic acid is 2.38%. Total polyunsaturated fatty acids were identified as the third-highest among fatty acids. The content of linoleic acid, a constituent of total polyunsaturated fatty acids, was measured at 17.62%, and the proportion of linolenic acid was determined to be 9.60%.

With the exception of *M. piperita*, all plant species exhibited a comparable profile in terms of polyunsaturated fatty acid composition, according to Sarikurcu et al. [33], who identified the primary fatty acids in the remaining plant species as linoleic (C 18:2 6) and -linolenic acids (C 18:3 3). *M. sylvestris* exhibited the greatest overall polyunsaturated fatty acid content (65.56%) of these species. *E. cicutarium* (63.23%) came in second place just behind it. Unsaturated fatty acids made up a large portion of this plant (73.12). It was discovered that the total unsaturated fatty acid concentrations of *M. sylvestris* and *C. draba* (72.08% and 71.14%, respectively) were too similar to those of *E. cicutarium*.

Mineral substance content of *Erodium cicutarium* (L.) L'Hér.

The mineral composition of *E. cicutarium* (L.) L'Hér. was determined and the obtained data is given in Table 6. While the most calcium, magnesium and potassium were found among the 9 different mineral substances determined, respectively; the lowest element was found to be nitrogen. Looking at the results, it was determined that the plant is a high source of calcium (1078.503 mg/kg).

Considering the cellular functions and the amount in the body, it is a known fact that potassium, calcium and magnesium are important mineral substances in the body. High potassium and low sodium diets are recommended for healthy eating habits. It may be possible to say that *E.*

Table 6 Mineral composition of *Erodium cicutarium* (L.) L'Hér.

Mineral								
Zn(mg/kg)	Fe (mg/kg)	Mn(mg/kg)	Cu(mg/kg)	K(mg/kg)	Na(mg/kg)	Mg(mg/kg)	Ca(mg/kg)	N(%)
88.076	11.046	14.107	71.895	629.043	127.287	994.918	1078.503	4.704

Table 7 Inhibition zone diameters (mm) of different extracts of *E. cicutarium* (100 mg/mL concentration, 20 µL) against tested bacteria (mm)

Tested microorganisms/Solvents	Methanol	Ethanol	Water	Diethyl ether	Methanol (NC)	Tetracycline (PC)	Polymyxin B (PC)
<i>E. coli</i> ATCC 25922	-	-	-	-	-	19	
<i>Proteus</i> spp.	-	-	-	-	-	9	
<i>P. aeruginosa</i> ATCC 9027	5.5	-	-	11	-		17

(-): Any inhibition zone was not observed on MHA plates. PC: Positive control, NC: Negative control

cicutarium (L.) L'Hér. is one of the plants that will meet these dietary requirements. In addition, Özdeştan and Üren [41] stated in their study that green leafy vegetables constitute the primary dietary source of nitrate, and the presence of nitrate in vegetables depends on nitrogen compounds in the soil. The researchers stated that when the nitrogen level in the soil is high, the vegetables grown in these regions also have high nitrate content, and the increase in nitrate and nitrite levels in foods will pose an important problem in terms of public health. When we look at the results of our study, it may be possible to say that nitrogen is the lowest mineral and therefore its consumption will not be harmful.

Bilic et al. [29] utilized inductively coupled plasma atomic emission spectrometry to determine the concentrations (µg/g) of eighteen elements (Al, Ba, Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni, P, Pb, S, Sr, and Zn) in *E. cicutarium* herb from four locations in Croatia (Buzin, Trešnjevka, Plitvice, Podvinje). The authors reported that the concentrations of the most abundant elements in *E. cicutarium* herb from the four localities followed this order: Mg > Ca > K > S > P > Na. The values for elements Mg, Ca, K, S, P, and Na ranged from 23140.0 to 46306.1, 20033.3 to 33565.7, 16815.0 to 19463.2, 4664.4 to 5978.4, 3072.1 to 5585.3, and 317.2 to 834.3, respectively.

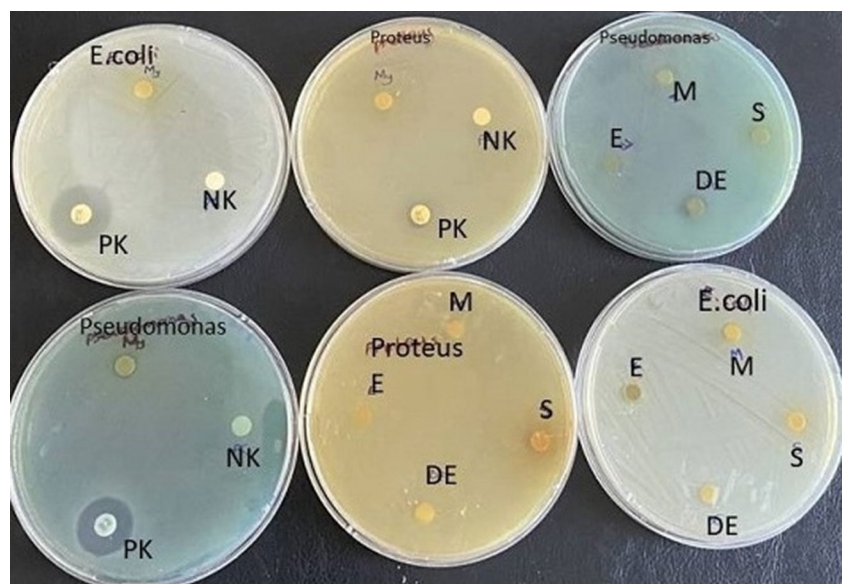
Antibacterial activity of different extracts of *Erodium cicutarium* (L.) L'Hér.

Ethanol and water extracts did not show antibacterial activity against test bacteria. *E. coli* ATCC 25922 and *Proteus* spp. exhibited greater resistance to all the herbal extracts (Table 7). The diethyl ether extract demonstrated the most substantial inhibition zones, with a zone diameter of 11 mm, against *P. aeruginosa* ATCC 9027. The inhibition effect of diethyl ether extract at 100 mg/mL concentration was lower than standard antibiotic (17 mm).

A methanol extract exhibited an inhibitory effect against *P. aeruginosa* ATCC 9027, with a zone diameter of 5.5 mm. Concentrated methanol was used as negative control and antibacterial activity of methanol was not found on isolates. The inhibition zone of the standard antibiotic Polymyxin B, used as a positive control for *P. aeruginosa* ATCC 9027, was measured as 17 mm. Compared to the reference antibiotic Polymyxin B, the measured zone diameter was lower; was found to have lower antimicrobial activity. Tetracycline antibiotic was used as positive control for *E. coli* ATCC 25,922 and inhibition zone was measured as 19 mm. Tetracycline *Proteus* spp. was also used for this and the zone diameter was measured as 9 mm (Fig. 1).

Nikitina et al. [42] investigated the antibacterial activity of plant extracts from the Geraniaceae and Rosaceae families, which included *E. cicutarium*. They found bacteriostatic

Fig. 1 Inhibitor effects of the different extracts of *Erodium cicutarium* (L.) L'Hér. against *E. coli* ATCC 25922, *Proteus* spp., *P. aeruginosa* ATCC 9027



activity higher in water extracts of *E. cicutarium* than those with ethanol. They explained this activity with the presence of polyphenols in the extract. Again in the same study, the most active water extract showed activity against 4 of the 11 *B. subtilis* strains tested and all 7 *Pseudomonas sp.* strains tested. It also supports our work; It is thought that the inhibitory effect of the methanol extract of *E. cicutarium* against *P. aeruginosa* the presence of polyphenols in the extract may account for this phenomenon can be explained by the presence of polyphenols in the extract.

α -Amylase and Tyrosinase inhibitory activities of different extracts of *Erodium cicutarium* (L.) L'Hér.

Table 8 shows the α -amylase and tyrosinase inhibitor activity results ($p < 0.05$). As can be seen from the Table 8, α -amylase inhibitor activity results ranged from 28.33 to 64.44%. While the lowest inhibition was observed in the water extract, the extract with the highest inhibition was the ethanol extract. In the tyrosinase inhibitor activity results, the highest inhibition was detected in the water extract with 50.14%; the lowest inhibition was detected in methanol extract with 38.18%.

Acarbose is one of the α -amylase inhibitory medicines that can contribute to the reduction of blood glucose levels by impeding the digestion of carbohydrates [43, 44]. Side effects of synthetic α -amylase inhibitors include flatulence, hypoglycemia, cholestasis, meteorism, and abdominal distention [44]. Therefore, to properly lower the blood glucose level, it is imperative to discover new and alternative natural agents with little to no negative effects [33, 40]. According to our amylase inhibitor activity results, ethanol extracts of *Erodium cicutarium* (L.) L'Hér may be preferred as an alternative natural agent to regulate blood sugar due to its amylase inhibitory activity.

The primary enzyme in the route leading to the manufacture of melanin in both plants and animals is tyrosinase. Tyrosinase activity can be inhibited to slow down melanogenesis, the process of synthesizing melanin pigment [45]. According to research, certain antioxidant molecules can block tyrosinase and regulate the process of melanogenesis

procedure [46]. Trolox, N-acetylcysteine, and vitamin C are among the antioxidant compounds that are actually employed to regulate the synthesis of melanin [47]. It would be reasonable to argue that plant species rich in antioxidants should be assessed primarily for their tyrosinase inhibitory capabilities, based on the notion that antioxidant molecules also give tyrosinase inhibition [48]. According to our tyrosinase inhibitor activity results, water extracts of *E. cicutarium* (L.) L'Hér. can be preferred as a natural agent due to tyrosinase inhibitor activity.

Conclusion

As can be seen from the data presented above, *E. cicutarium* (L.) L'Hér. showed strong antioxidant activity in almost all test systems. In general, methanol extracts exhibited quite strong antioxidant activities in the antioxidant analyzes. The extraction in which the most phenolic compounds were obtained was the methanol extraction. According to this result, it may be possible to say that best extraction for extracting phenolic compounds is methanol extraction. Our findings indicated that methanol extraction was adequate for protecting these components. Our results regarding the antioxidant activity of the extract indicate the importance of its use as a valuable food product and traditional medicine of *E. cicutarium*. From the results of present studies it can be safely concluded that the presence of appreciable amount of antioxidant activity, phenolic compounds, fatty acid and mineral composition in the plant might be useful for the food, healthy and pharmacology. In addition, future studies should also investigate the use of advanced extraction methods and the potential use of the herbal as food ingredients.

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Author contributions FUT: determination of the topic, writing of the article and carrying out analyzes; GK: writing of the article and carrying out analyzes; FES: carrying out antioxidant analyzes and extraction processing.

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Declarations

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Table 8 Inhibitory activities on tyrosinase and α -amylase of water, ethanol, methanol and diethyl ether extracts of *Erodium cicutarium* (L.) L'Hér.

Extracts (2 mg/mL)	Tyrosinase	α Amylase
E _{Water}	50.14 $\pm$ 2.31 ^a	28.33 $\pm$ 0.51 ^d
E _{Ethanol}	41.18 $\pm$ 4.14 ^b	64.44 $\pm$ 1.08 ^a
E _{Methanol}	38.18 $\pm$ 4.02 ^b	36.51 $\pm$ 0.61 ^c
E _{Diethyl ether}	42.43 $\pm$ 0.81 ^b	46.89 $\pm$ 0.71 ^b

*(The data shown are the mean values of $n = 3$. The difference between the values expressed with different symbols (a-b; a-d) in the same columns in the graph was significant ($p < 0.05$))

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