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Determination of Chemical Compositions, Antioxidant and Enzyme Inhibitory Activities of Naturally Growing *Chenopodium album* subsp. *iranicum* Aellen

Ayça AKTAŞ KARAÇELİK[1*], Hüseyin ŞAHİN[2]

ABSTRACT: *Chenopodium album* has been used as folk medicine and nutrition for years by local people. This study aimed to investigate the phenolic compounds by Reversed Phase-High Performance Liquid Chromatography-Diode Array Detector (RP-HPLC-DAD), total phenolic content (TPC), and biological activities of the plant extracts prepared with three solvents (methanol, acetonitrile, and water) for the first time. Also, the chemical composition of essential oil and mineral content of the plant were determined by Gas Chromatography Mass-Spectrometry (GC-MS), and Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES), respectively. The extracts of the plant were analyzed for the *in vitro* inhibitory activities against carbonic anhydrase (CA) and urease enzymes. The most abundant compound in methanol extract was catechin (157.666 mg L^{-1}). Moreover, depending on the solvent used in the extracts, varied levels of phenolic acids such as gallic, protocatechuic, *p*-OH benzoic, ferulic, and syringic acids were identified. Thirty-four components were identified and methyl linolenate was found to be the main constituents of the essential oil (11.28 %). The methanol extract of the plant exhibited the best antioxidant value given as TPC 65.41±5.20 µg mL^{-1} GAE, FRAP 113.54±1.57 µM TEAC, DPPH• 191.1±3.55 µg mL^{-1} SC_{50}, and $ABTS^{\bullet+}$ 74.7±1.55 µg mL^{-1} SC_{50}, respectively. According to the mineral analyses, it was conducted that potassium and sodium were the most abundant minerals. The extracts were found as inactive against CA and showed a moderate urease inhibition effect (IC_{50}: 28.380±0.742 mg mL^{-1}). The obtained results indicated that the plant extract could be used as an easily available natural antioxidants source for the food and pharmaceutical industry.

Keywords: *Chenopodium album* L., RP-HPLC-DAD, GC-MS, antioxidant, essential oil, minerals

[1] Ayça Aktaş KARAÇELİK (**Orcid ID:** 0000-0001-5381-2924), Department of Food Processing, Espiye Vocational School, Giresun University, Giresun, Turkey
[2] Hüseyin Şahin (**Orcid ID:** 0000-0002-6018-1494), Giresun University, Espiye Vocational School, Giresun, Turkey

***Sorumlu Yazar/Corresponding Author:** Ayça Aktaş KARAÇELİK, e-mail: ayca.aktas@giresun.edu.tr

INTRODUCTION

In recent years, herbal products have been gaining interest to heal various diseases and provide better living conditions in many countries. Especially, green leafy vegetables are of widespread interest among scientists because they offer many benefits for human health. There are many local and wild vegetables throughout the globe. Turkey has very important geographical features in terms of wild plants. *Chenopodium album* L. is commonly known as Salmanca in Iğdır. This plant belongs to the genus of *Chenopodium* and the family of Spinachaceae which is distributed worldwide (Nowak et al., 2016). The nutritional value of this plant, also known as wild spinach, has been found to be derived from proteins, lipids, minerals, carotenoids, and vitamin C (Pradhan et al., 2015; Samancioglu et al., 2016). Also, previous phytochemical studies have shown that *Chenopodium* species contain several secondary metabolites such as organic acids (Guil et al., 1996), phenol and flavanoids (Nahar and Sarker, 2005; Cutillo et al., 2006), coumarins (El-Sayed et al., 1999), catechins (Penarrieta et al., 2008). Many studies have been reported about antioxidant, antibacterial, antifungal, and anticancer abilities of many species of *Chenopodium* from different regions (Samancioglu et al., 2016; Nowak et al., 2016; Khomarlou et al., 2017; Külcü et al., 2019). The fatty acid profile of *C. album* leaves was identified as myristic acid, cis-10-pentadecanoic acid, and α-linolenic acid (Guerrero and Torija, 2009; Yılmaz et al., 2019). In recent studies, it has been discovered that the vegetables inhibit enzymes causing some metabolic diseases due to their bioactive components such as phenolic acids, phenols, polyphenols, and flavonoids (Laurenço et al., 2019). *C. album* L. has been traditionally used as antihelmintic, antiparasitic, antiseptic, diuretic, sedative, and hepatoprotective (Kokanova-Nedialkova et al., 2009). The new natural compounds to be identified will be important in the discovery of compounds with drug potential. It has been thought that *C. album* growing naturally around Iğdır is not given the necessary importance. More study is needed to determine the nutritional value and medicinal effects of this plant. It has been also thought that these studies will contribute to local agriculture.

In this connection, the aim of the current study was to evaluate the qualitative and quantitative analysis of bioactive phenolic components by RP-HPLC-DAD, the total phenolic content, and the antioxidant activity of the extracts prepared by using different solvents (methanol, acetonitrile, and water) of *Chenopodium album* subsp. *iranicum* Aellen growing in Iğdır province Eastern Anatolia region of Turkey. Inhibition effects of the water extracts on carbonic anhydrase and urease enzymes were also studied, since new natural and biocompatible inhibitors to be identified would be important in the discovery of compounds with drug potential. In addition, the essential oil and minerals composition of the plant were also determined by GC–MS and ICP-OES, respectively. As far as we know, this is the first detailed research about the determination of chemical composition, antioxidant, and some enzyme inhibition effects of *C. album* subsp. *iranicum* Aellen.

MATERIALS AND METHODS

Chemicals

2,4,6-tripyridyl-s-triazine (TPTZ), anhydrous iron (III) chloride ($FeCl_3$), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH•), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), butylated hydroxytoluene (BHT), jack bean urease, bovine carbonic anhydrase (bCA), *p*-nitrophenyl acetate (*p*-NPA), HNO_3, H_2O_2, and all phenolic standards were purchased from Sigma–Aldrich. Folin-Ciocalteu reagent, sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), and sodium acetate ($C_2H_3NaO_2$) were supplied from Merck. All solvents were HPLC grade and obtained from Merck.

Plant Material and Sample Preparation

Plant was collected in the second half of June and various days of August 2019 from their natural environments in Iğdır Province in the Eastern Anatolia Region of Turkey. Locally known as Salmanca plant was identified as *Chenopodium album* subsp. *iranicum* Aellen by Assoc. Prof. Dr. Mutlu Gültepe (Department of Crop Production and Technology, Giresun University, Turkey).

Plant was washed first with running tap water and then with distilled water. Fresh leaves of plant were dried in an oven at 40 °C until a constant mass was reached. Subsequently, a metal blender was used to grind leaves before solvent extraction. 10 g of dried sample was added to flask with 100 mL of different extraction solvent (methanol, acetonitrile, and water) and extracted on a magnetic stirrer at 200 rpm at 40 °C for 4 hours. Extracts were centrifuged at 2500 rpm for 30 min to obtain a clear solution, then passed through a blue band filter paper, and then filtered through a membrane filter (0.45 µm syringe filters, Whatman). Final concentrations of methanol (15 mg mL^{-1}) and acetonitrile (1 mg mL^{-1}) extracts were concentrated using a rotary evaporator (Heidolph, Germany) at 40 °C, water extract (25 mg mL^{-1}) was lyophilized. Finally, all samples were stored as a small volume aliquant (2 mL) at -18°C for further analysis. Essential oil was hydrodistilled from milled plant using a Clevenger-type apparatus for 5 hours. Essential oil was then dehydrated with anhydrous sodium sulfate and stored at +4°C for further analyses.

Phenolic Compound Analysis by RP-HPLC-DAD

Eighteen phenolic standards (benzoic acid, caffeic acid, catechin, chlorogenic acid, epicatechin, ferulic acid, gallic acid, protocatechuic acid, protocatechuic aldehyde, *p*-OH benzoic acid, *p*-coumaric acid, syringaldehyde, syringic acid, rosmarinic acid, rutin, vanillic acid, vanillin, and quercetin) were used in this study by comparison at 4 different wavelengths (260, 280, 308, and 324 nm by DAD). Analysis was performed by using RP-HPLC-DAD (Thermo Scientific, Bremen, Germany) instrument and reverse phase C_{18} column (150 mm x4.6 mm, 5µ; Fortis). 2% acetic acid-ultra-pure water in reservoir A and 50-50% acetonitrile-ultra-pure water including 0.5% acetic acid in reservoir B was applied with a gradient program: 0-10 min (5% B), 10-20 min (10% B), 20-25 min (25% B), 25-28 min (38% B), 28-35 min (43% B), 35-37 min (50% B), 37-39 min (90% B), 39-43 min (20% B). Mobile phase flow rate was set to 0.7 mL min^{-1}, injection volume to 20 µL, and column temperature to 25 °C. Limit of Detection (LOD) and Limit of Quantification (LOQ) known as validation parameters were calculated for each standard according to the signal/noise (S/N) level of 3 and 9, respectively (Table 1).

Table 1. RP- HPLC-DAD validation parameters

No	Compound	RT_{mean} (min)	m[a]	R^2	LOD[b]	LOQ[b]
1	Gallic Acid	7.15	1.0597	0.9973	0.0316	0.0957
2	Protocatequic Acid	13.04	2.0259	0.9965	0.0097	0.0293
3	Protocatequic Aldehyde	19.20	0.6452	0.9961	0.1070	0.3242
4	*p*-OH Benzoic Acid	20.61	1.6158	0.9969	0.0156	0.0472
5	Catechin	22.17	4.6834	0.9974	0.0019	0.0057
6	Chlorogenic acid	22.91	2.1609	0.9959	0.0080	0.0241
7	Vanillic Acid	24.87	1.5219	0.9964	0.0153	0.0464
8	Caffeic Acid	25.58	1.0534	0.9993	0.0426	0.1292
9	Syringic Acid	26.75	1.0517	0.9974	0.0393	0.1191
10	Epicatechin	27.82	4.2632	0.9971	0.0022	0.0066
11	Vanillin	30.03	0.8083	0.9964	0.0780	0.2362
12	*p*-Coumaric Acid	31.40	0.7531	0.9989	0.0961	0.2913
13	Syringaldehyde	31.72	2.3812	0.9985	0.0117	0.0354
14	Rutin	32.90	4.6524	0.9921	0.0065	0.0198
15	Ferulic Acid	33.51	1.1052	0.9947	0.0307	0.0930
16	Benzoic Acid	38.24	7.4800	0.9982	0.0013	0.0039
17	Rosmarinic Acid	39.66	2.2325	0.9972	0.2363	0.7162
18	Quercetin	42.52	2.2321	0.9967	0.0331	0.1003

[a]: slope, [b]: values were given in mg L^{-1}

Antioxidant Activity

ABTS•$^{+}$ radical scavenging capacity assay

ABTS•$^{+}$ radical scavenging capacity in all extracts of *C. album* subsp. *iranicum* Aellen was measured with a spectrophotometric method which was measured at 734 nm (Re et al., 1999). The results were expressed as SC_{50} values (the sample concentration that provides 50% scavenging of ABTS•$^{+}$ radical, μg mL^{-1}).

DPPH radical scavenging activity assay

In this assay, the procedure of DPPH• radical scavenging activity stated by Brand-Williams et al., (1995) was applied with some minor modifications. After measuring the absorbance at 517 nm, all results were calculated as SC_{50} values (μg mL^{-1}). The tests were carried out thrice. Trolox and BHT were used to compare the results. A low SC_{50} value indicates high antioxidant activity (Brand-Williams et al., 1995).

Ferric reducing/antioxidant power (FRAP) assay

FRAP values of all extracts of *C. album* subsp. *iranicum* Aellen were determined using the actual method in the literature (Benzie and Strain, 1999). Also, Trolox as the standard was tested with this current methodology at 595 nm. Thus, all values were expressed as micromolar Trolox equivalent antioxidant capacity (TEAC).

Total Phenolic Content (TPC)

The total phenolic contents in *C. album* subsp. *iranicum* Aellen extracts were determined according to a spectrometric assay (Slinkard and Singleton, 1977). After the addition of relevant ingredients introduced by the current methodology, the absorbance was measured against a blank at 765 nm. A calibration curve was prepared using a standard solution of gallic acid and the results were expressed as microgram gallic acid equivalents per milliliter sample (μg GAE mL^{-1} sample).

Gas Chromatography-Mass Spectrometry (GC–MS) Analysis

Thermo Scientific Brand GC-MS was used to identify the chemical composition of the extracts. Analytical separations were quantified using the TG-5MS column (30 m, 250 μm ID, 0.25 μm df). The analyses were carried out by specified conditions: the flow rate of carrier gas (Helium): 1.0 mL min^{-1}, injection port temperature: 250°C, the ionization mode: 70 eV, initial temperature: 50°C, and oven temperature 220°C for 0.67 min for the oven. The oven temperature was programmed to 250°C at a rate of 5°C/min and then kept constant at 250°C for 5 min. The components were determined by comparison of their relative retention times and mass spectra with those of standards, according to literature (Adams, 2007) and available on database Wiley and NIST library.

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) Mineral Analysis

The samples were decomposed by the microwave digestion system (Berghof Instruments, Speedwave, Germany). 0.3 g of each sample was added into 5 mL of 65% (m/v) HNO_3 solution with 2 mL of 35% (m/v) H_2O_2 solution. The samples were operated at 145 °C for 5 min, 200 °C for 10 min, and 50 °C for 10 min for microwave digestion. After one hour, colorless solutions were completed to 25 mL with deionized water.

Total concentrations of Ca, Fe, Mg, P, Zn, Cu, and Na were determined by the ICP-OES apparatus (Thermo Scientific iCap 6000 Dual view, Thermo Scientific, Cambridge, England). Total concentrations of K were determined by the AAS apparatus (Thermo Scientific iCE 3000 Series, Thermo Scientific, Cambridge, England). Cu, Ca, Fe, Mg, P, and Zn were prepared by using dilutions of a multi-element

(100 mg mL^{-1}) ICP QC standard solution, K standard (1000 mg mL^{-1}), and Na standard (1000 mg mL^{-1}) as standards for calibration system. Analytical lines of Ca 317.9 nm, Fe 259.9 nm, Mg 279.5 nm, P 177.4 nm, Zn 213.8 nm, Na 589.5 nm, Cu 324.7 nm, and K 766.5 nm were also measured. LOQ of the elements were calculated as Ca (1.1 ppm), Fe (0.4 ppm), Mg (0.6 ppm), P (0.3 ppm), Zn (0.3 ppm), Na (0.8 ppm), Cu (0.2 ppm), and K (0.1 ppm) (Sezer et al., 2018).

Carbonic Anhydrase and Urease Inhibition Assays

The inhibition effect of water extract of the plant on carbonic anhydrase was carried out according to a method modified by Verpoorte et al. (Verpoorte et al., 1967). The IC_{50} value was determined by measuring CA esterase activity. 750 µL of *p*-nitrophenyl acetate (3 mM) was added to the mixture containing 550 µL 0.05 M Tris-SO_4 buffer (pH 7.4), 150 µL enzyme solution, 50 µL sample solutions and vortexed. After 30 min, the mixture absorbances were recorded at 348 nm. The results were evaluated in comparison with two controls by preparing without enzyme and sample solution. All measurements were recorded in triplicate. The activity (%)-inhibitor concentration [I] graphs were drawn for each sample that had an inhibitory effect on the bCA enzyme, and inhibitor concentration (IC_{50}) values that caused 50% inhibition were calculated.

The measurement of urease inhibition activity in water extract was built on the determination of the ammonia product under the indophenol method (Weatherburn, 1967). In summary, 200 µL of jack bean urease, 500 µL of buffer (100 mM urea, 0.01 M K_2HPO_4, 1 mM EDTA, and 0.01 M LiCl, pH 8.2) and 100 µL of the extract was mixed and kept at room temperature for 20 minutes. At the end of the incubation period, phenol reagent (550 µL, 1% w / v phenol and 0.005% w / v sodium nitroprusside) and alkaline reagent (650 µL, 0.5% w / v sodium hydroxide and 0.1% v / v NaOCl) were mixed. The changing absorbance values after 50 minutes were measured at 625 nm with a spectrophotometer (Shanghai Mapada Instruments Co., China).

Statistical Analysis

The results were statistically analyzed by one-way ANOVA followed by the Duncan test using SPSS 17.0 Inc. software version. The data were given as mean ± standard error (SD) of three experiments. The same letters in each column were not significantly different at $P < 0.05$.

RESULTS AND DISCUSSION

Identification of Phenolic Compounds in *C. album* Extracts

A major part of people relies on some natural products for the treatment of some diseases due to the claiming to be the definitive or palliative solution against the current diseases. Sometimes, this claim should be considered because of some results of the negative properties of drugs such as biological side effects. Plants are a significant member of these natural products, these are known as a source of nutrition, cosmetic, and medicine (Che and Zhang, 2019) by humans because these natural products have valuable bioactive compounds that are responsible for significant activities such as antioxidant, anti-inflammatory, and antimicrobial, *etc*. The phenolic compounds are well known as secondary metabolites are responsible for many bio-properties (Afolayan and Jimoh, 2009). *C. album* subsp. *iranicum* Aellen is one of the natural products frequently consumed by local people. The current study includes many analyses to evaluate the different results in terms of chemical composition and bioactivity perspective. Also, according to our knowledge, this is the first report about phenolic compounds defined by RP-HPLC-DAD in *C. album* subsp. *iranicum* Aellen extracts prepared with different solvents (methanol, acetonitrile, and water). The relevant results are presented in Table 2. In this study, RP-HPLC-DAD has used a method that is developed and validated to identify the phenolic compounds found in *C. album*

extracts. Phenolic compounds of three extracts were determined by the retention time and UV spectra. Moreover, these were compared with the previously reported standards in the literature. When considered in terms of the characterization of the flavonoids, the most abundant compound was catechin is a part of the family of flavonoids with 157.666 mg L^{-1} in methanolic extract. Although it was studied with eighteen phenolic compounds, six of them were detected with the level of mg per L. Moreover, it was seen that the general profile was formed with the members of the phenolic acids which were identified as gallic acid, protocatechuic acid, *p*-OH benzoic acid, ferulic acid, and syringic acid. Amic et al. (2003) and Afolayan and Jimoh (2009) claimed that flavonols and flavonoids had significant value to revealed antioxidant and free radical scavenging activity in natural compounds. The previous studies have suggested that methanolic extracts contain more phenolic compounds than the other extracts obtained by different solvents and have the highest antioxidant value (Chigayo et al., 2011; Lim et al., 2019). Cutillo et al. (2006) isolated and identified some components as phenols and lignans of *C. album*. Even though their results had a wide range of scales, it was good news due to the correlation with our results that the ferulic acid was identified. In another study, besides ferulic acid, protocatechuic acid was also determined in the methanolic extract of the *C. album* (Laghari et al., 2011). When evaluated all literature data including our results, the differences of the phenolic constitute in the *C. album* could be normal because the variation of the geographically, climatically, and the sub-species are important to the phenolic characterization (Adedapo et al., 2011; Efe, 2020). According to previous studies, there is an extremely positive relationship between total phenols and the antioxidant activity of many plant species. As the phenolics are important components of this plant, the antioxidant effects could be attributed to the presence of these valuable constituents (Penarrieta et al., 2008; Samancioglu et al., 2016).

Table 2. Analysis of phenolic compounds of different extracts of *C. album* subsp. *iranicum* Aellen by RP-HPLC-DAD (ppm, mg L^{-1} extract)

Phenolic compounds	Methanol	Water	Acetonitrile
Gallic acid	0.272	N.D.	N.D.
Protocatechuic acid	0.514	N.D.	N.D.
p-OH benzoic acid	N.D.	0.866	N.D.
Catechin	157.666	N.D.	N.D.
Ferulic acid	10.65	N.D.	N.D.
Syringic acid	N.D.	0.535	N.D.

N.D: Not Detected

Antioxidant Activity and Total Phenolic Content of *C.album* Extracts

The antioxidant capacities of three extracts were evaluated by DPPH•, ABTS•$^{+}$, and FRAP. For all methods, the best value was seen in methanol extract given as TPC 65.41±5.20^{a} µg mL^{-1} GAE, FRAP 113.54±1.57^{a} µM TEAC, DPPH• 191.1±3.55^{b} µg mL^{-1} SC_{50}, and ABTS 74.7±1.55^{b} µg mL^{-1} SC_{50}, respectively (Table 3).

The results of this study which were underlined the yield of methanolic extraction affirmed with other plant studies focused on different solvents (Karaçelik et al., 2015). The other organic solvent extraction was realized with acetonitrile had a better value than aqueous extraction. Interestingly, the activity of the scavenging of the ABTS•$^{+}$ radical (76.3±4.25^{b} µg mL^{-1}) was nearly the same as the methanolic extract (Table 3). A positive correlation (R^2=0.667) between TPC and FRAP antioxidant activity was detected, while strong positive correlations (R^2=0.907 for DPPH• and R^2=0.866 for ABTS•$^{+}$) were observed between TPC and radical scavenging tests. The highest antioxidant activity was detected in methanol extract and the lowest result was detected in the water extract. The high linear correlation coefficient (R^2=0.982) was determined between DPPH• and ABTS•$^{+}$ methods. In addition,

the results obtained from TPC and antioxidant tests are parallel to the literature (Penarrieta et al., 2008; Samancioglu et al., 2016).

Table 3. Antioxidant activities and total phenolic content of three extracts of *C. album* subsp. *iranicum* Aellen[a] and some chemical standards

Extracts	ABTS•+ (SC_{50}, µg/mL)	DPPH• (SC_{50}, µg/mL)	FRAP (µM, TEAC)	TPC (GAE, µg/mL)
Methanol	74.7±1.55[b]	191.1±3.55[b]	113.54±1.57[a]	65.41±5.20[a]
Water	103.6±1.90[c]	664.4±0.85[d]	23.75±1.87[c]	15.65±1.63[c]
Acetonitrile	76.3±4.25[b]	499.3±16.45[c]	68.33±3.44[b]	30.55±3.59[b]
BHT	0.5±0.02[a]	8.4±0.20[a]	N.D	N.D
Trolox	2.3±1.72[a]	3.0±0.02[a]	N.D	N.D

[a]: Each value represents the average of three repetitions. The same letters in each column were not significantly different at $P < 0.05$ (Duncan's multiple range test). Means are given with standard deviations; N.D: Not Detected

The Chemical Composition of Essential Oil of *C. album*

The qualitative and quantitative analysis of the chemical composition of the essential oil of *C. album* subsp. *iranicum* Aellen was determined by GC-MS and given in Table 4. Thirty-four components were identified in terms of the database in the current equipment library. The methyl linolenate, which was a polyunsaturated fatty acid methyl ester that derived from alpha-linolenic acid, tagged as the main constituents of the current sample with the 11.28 % area.

In extensive research based on the determination of the fatty acid of *C. album* was determined alpha-linolenic acid was the dominant (Guerrero and Torija, 2009). In another report about different subspecies of *C. album*, the main component of the fatty acid was determined as myristic acid (18.26%) and cis-10-pentadecanoic acid (15.39%) (Yılmaz et al., 2019).

Also, Khomarlou et al. (2018) claimed that the *C. album* subsp. *striatum* was a bioactive potent source due to its effective compounds such as phytol, linalool, alpha-terpeneol, and linolenic acid. In terms of the current study's result, the major and minor components of the essential oil of *C. album* differ from the same plants collected from different geographical regions. These differences could be attributed to climatic conditions, collecting season, water stress, and experimental conditions of the plants (Khomarlou et al., 2018; Yılmaz et al., 2019).

Table 4. The results of GC-MS of *C. album* subsp. *iranicum* Aellen

No	RT	Area%	Name
1	11.426	0.24	Benzeneacetaldehyde
2	17.376	0.34	Safranal
3	18.235	0.40	beta-Cyclocitral
4	19.368	0.22	Naphthalene
5	19.574	0.25	1-Cyclohexene-1-acetaldehyde, 2,6,6-trimethyl-
6	20.981	0.39	1-Oxaspiro[4.5]dec-6-ene, 2,6,10,10-tetramethyl-
7	22.732	1.31	Naphthalene, 1,2-dihydro-1,1,6-trimethyl-
8	23.751	1.31	beta-Damascenone
9	24.952	0.27	alpha-Ionone
10	25.628	0.40	5,9-Undecadien-2-one, 6,10-dimethyl-(E)
11	26.818	6.38	3-Buten-2-one, 4-(2,6,6-trimethyl-1-cyclohexen-1-yl)-
12	28.420	0.40	1,3,5-Trimethyl-2-cyclopentylbenzene
13	28.958	0.80	Megastigmatrienone 4
14	29.558	1.62	Megastigmatrienone 2
15	30.497	0.43	(Z)-4-[(E)-(But-2-enylidene]-3,5,5-trimethylcyclohex-2-enone
16	30.926	1.62	Benzophenone
17	31.870	0.89	Cyclooctasiloxane
18	33.427	1.34	Methyl tetradecanoate
19	33.673	0.55	7-Tetradecene
20	36.545	2.22	2-Pentadecanone
21	37.546	2.79	7,10,13-Hexadecatrienoic acid, methyl ester

Table 4. The results of GC-MS of *C. album* subsp. *iranicum* Aellen (continued)

No	RT	Area%	Name
22	38.233	7.79	Hexadecanoic acid, methyl ester
23	38.513	0.85	Isophytol
24	38.851	0.83	Ethyl linolenate
25	39.395	1.72	Ethyl palmitate
26	39.927	0.34	Methyl 10-methyldodecanoate
27	40.871	1.10	Dibutyl isophthalate
28	41.678	11.28	Methyl linolenate
29	42.038	8.78	Phytol
30	42.330	1.13	(3E,5E,7E)-5-Methyl-8-(2,6,6-trimethyl-1-cyclohexene-1-yl)-3,5,7-octatriene-2-one
31	42.570	0.45	Linoleic acid ethyl ester
32	42.730	1.48	Ethyl 9,12,15-octadecatrienoate
33	44.819	0.28	Tricosane
34	53.831	1.80	n-Docosane

Mineral Contents of *C. album*

According to the mineral contents (Table 5), it was conducted that potassium and sodium were the most abundant minerals throughout the fifteen elements. Hence, these were tagged as macronutrients. When compared to previous studies in terms of the analysis of the mineral content of the subspecies of *C. album*, a high similarity was seen in our results. Afolayan and Jimoh (2009) confirmed that potassium was dominant like our data (Afolayan and Jimoh, 2009). In another study from Saudi Arabia -except for calcium because it had the highest value- many analyzed elements showed nearly high harmony with our data (Daur, 2015). Another similar observation was declared by Pradhan et al. (2015) who underlined potassium level as the dominant. But they also reported that sodium was 7.4 mg 100 g^{-1} as a moderate, unlike our result (Pradhan et al., 2015). It has been well-known that the mineral content of the plants could change with soil composition and the rate of uptake of minerals by plant species (Akpanyung, 2005; Pradhan et al., 2015).

Table 5. Mineral contents of *C. album* subsp. *iranicum* Aellen (mg kg^{-1}, ppm)

Elements	ppm
Macro Elements	
Potassium	62118.8
Sodium	34285.9
Magnesium	8679.6
Phosphorus	7253.9
Calcium	2160.7
Iron	354.7
Aluminum	322.3
Micro Elements	
Manganese	49.2
Copper	11.4
Nickel	3.4
Chrome	1.3
Arsenic	0.8
Cobalt	0.3
Lead	0.095
Mercury	<0.0

Carbonic Anhydrase and Urease Inhibition Effects of *C. album*

The urease is an enzyme found in various bacteria, fungi, and plants and hydrolyzes urea to carbon dioxide and ammonia (Konieczna et al., 2012; Upadhyay, 2012). Carbonic anhydrase is an important enzyme that catalyzes the reversible hydration reaction of carbon dioxide to bicarbonate (Koutnik et al., 2017). The inhibition and activation effects of many enzymes (also included these mentioned enzymes) by drugs and chemicals have been investigated by scientists and reported in the literature (Supuran,

2008; Supuran et al., 2008; Upadhyay, 2012). Discovering new inhibitory and activatory compounds has become very important in recent years. To the best of our knowledge, this study is the first report on *in vitro* bovine carbonic anhydrase (bCA) and urease enzyme activity of *Chenopodium* species. In the light of this information, it was aimed to try two enzyme inhibition assays such as bCA and urease with the water extract of *C. album*. According to the *in vitro* enzyme inhibition results, the inhibition effect of the water extract on the urease enzyme was found to be quite low (IC_{50}: 28.380 ±0.742 mg mL^{-1}), while no inhibition on the bCA enzyme was found.

CONCLUSION

Natural products are a significant study field in complementary medicine thanks to their impressive bioactive compounds. Generally, these studies are performed on a wide and different scale. In light of this reality, it was aimed to evaluate a sub-species of *C. album* with many *in vitro* analyses based on the bio-activity approach. When compared with the literature data, the sample studied in this study sample did not exhibit effective results in terms of the studied phenolic standards and enzymes. It is believed that more investigations are needed to increase the scientific value. Even though this sample has limited numbers of phenolic compounds, it is known that these are considerable compounds. Moreover, preclinical studies can be designed due to high-level mineral potential.

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Conflict of Interest

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

Author's Contributions

The authors declare that they have contributed equally to the article.

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