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Determination of Multi-Elemental Analysis and Antioxidant Activities of *Helichrysum arenarium* (L.) Moench Species

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ABSTRACT

In this study, multi-elemental analysis and antioxidant activity of samples belonging to two different populations of *Helichrysum arenarium* (L.) Moench known as the immortal flower was determined. The results obtained were compared among themselves. Ca, Mg, Al, Fe, Na, Li, Be, B, Ti, Cr, Ni, Zn, Mo, and Pb element contents of the sample belonging to the B population were determined that be higher than that of the other population. The K, V, Mn, Co, Cu, As, Se, Sn, and Ba element contents of the sample belonging to the G population were determined that be higher than that of the other population. When the antioxidant activity results of the species were evaluated; according to the CUPRAC (Cupric Reducing Antioxidant Capacity) method, the sample extracts of the B and G populations were determined that showed lower activity than the standard BHA, BHT, and Trolox values at 20 and 40 µg/mL concentrations, and showed close activity compared to the standards at 80 µg/mL concentrations. The species was determined that be antioxidant activity even at low concentrations. According to DPPH (2,2-diphenyl-1-picrylhydrazyl) method, the antioxidant activity of the extract of the B and G populations was determined as 22.95 and 23.76 mg TE/mL, respectively.

Keywords: *Helichrysum arenarium,* Antioxidant, Bivariate analysis, ICP-MS

1. Introduction

The name *Helichrysum* is derived from the Greek words "Helios" meaning sun and "Chrysos" meaning gold [1]. *Helichrysum arenarium* (L.) Moench is a perennial herbaceous species belonging to the Asteraceae family. Among the people, it is known as golden grass, lasting forever, immortal flower or never fade flower [1-7]. There are over 600 species of *Helichrysum* genus in the world. Species of this genus are distributed over a wide geographical area, including America, Scandinavia, Atlantic, Europe, the Balkans, Russia, Siberia, the Caucasus, Asia Minor, Central Asia, Mongolia, and China. *H. arenarium* is a perennial species that grows in the steppes, on sandy and semi-hard soils, and grows to a height of 90 cm [8–10]. The genus *Helichrysum* is commonly found in Anatolia and represented in the flora of Turkey by 27 taxa, 15 of which are endemic [3].

According to pharmacological data, *H. arenarium* species flower is rich in phenolic compounds, including flavonoids, essential oils, fatty acids, carotenoids, steroids, polyphenols, vitamins, mineral salts, polysaccharides, glycosides, coumarins, catechins, and proanthocyanidins. Also, it contains components such as astragalin, luteolin, kaempferol, etc. [1,4,8,11].

Flavonoids are considered the main components responsible for the antioxidant properties of the plant [12]. Also, they exhibit pharmacological effects due to their phenolic structure and inhibition of free radical-mediated processes. Free radicals play an important role in many pathological conditions. The use of synthetic antioxidants to prevent free radical damage has been reported to have toxic side effects. Thus, there has been a growing interest in natural antioxidants and scavenger natural compounds [13]. Synthetic antioxidants such as tert-butylated hydroxytoluene (BHT), tert-butylated hydroxyanisole (BHA), etc. are often not preferred as preservatives in the pharmaceutical and food industry. Therefore, the use of safe natural antioxidants gets more attention [12].

The flower of this species has many biological activities, especially hepatoprotective, cholinergic, antibacterial, antiviral, antifungal, anti-inflammatory, antiproliferative, antimicrobial, antiallergic, antioxidant, and antiradical [1,14,15]. It has been used in folk medicine to treat various ailments such as liver and gallbladder ailments, lumbago treatment, stomach pain, asthma, arthritis disorders, cystitis and jaundice, skin infections, respiratory and digestive system disorders, kidney stones, and urogenital disorders. It has also been used for many years in the cosmetic industry for its fragrance [1]. It is widely used as herbal tea in Turkey [4]. It is used in Southern Africa to treat tuberculosis and related symptoms, and traditionally in Central Europe as antiseptic and spasmolytic drugs [6,9,16,17].

The World Health Organization (WHO) states that the maximum allowable concentration levels in raw plant materials for cadmium, arsenic, and lead are 0.3, 1.0, and 10 mg/kg, respectively [18,19]. Various methods are used to determine element content and concentrations in species samples. These methods are Atomic Absorption Spectrometry (AAS), Flame Atomic Absorption Spectrometry (F-AAS), Graphite Furnace Atomic Absorption Spectrometry (GF-AAS), Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES), Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES), Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and X-ray Fluorescence Spectrometry (XRF) [20–24].

In this study, multi-elemental analysis, antioxidant activities, and bivariate data analysis of *H. arenarium* species belonging to two different populations were performed and compared. Also, the obtained As, Cd, and Pb metal content analysis results were compared with the WHO's maximum allowable concentration values in raw plant materials.

2. Material and Methods

2.1. Plant materials

Two different populations of the *H. arenarium* were used in the study. These specimens were collected from Bitlis Nemrut Crater Lake ((38° 37′ 10″ N), (42° 14′ 28″ E), altitude 2.628 m)) (B) and the mountainous areas of Sebinkarahisar District of Giresun ((40° 23′ 34″ N), (38° 18′ 33″ E), altitude 1.569 m)) (G) during the flowering period between June and August 2020. Samples belonging to the species were kept in Mardin Artuklu University Herbarium. The taxonomic identification of the species was confirmed by the botanists of the same institution. Species were dried in the shade and areas with airflow and stored for multi-elemental analysis by ICP-MS.

2.2. Reagents and solutions

Analytical purity HNO_3 (70% (Sigma Aldrich, Germany) and ultrapure water (18.2 MΩ) were used in

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the microwave solubilization process. Ag, Al, As, B, Ba, Bi, Ca, Cd, Co, Cr, Cu, Fe, K, Li, Mg, Mn, Mo, Na, Ni, Pb, Sb, Se, Sn, Sr, Ti, Tl, V and Zn as the mixed standard (100 mg/L, Plasma CAL Calibration solution SCP28AES) in ICP-MS measurements were used. Calibration standard solutions (1-2000 µg/L) were prepared by appropriate dilution of stock mix standards (1 mg/L). The linear range, regression, correlation coefficient (R), limit of detection (LOD), and limit of measurement (LOQ) values of the calibration chart are shown in Table 1. LOD and LOQ values for twenty-four metals were calculated using 10 independent blank solutions. Analytical purity methanol (75%), $CuCl_2$, neocuprin, CH_3COONH_4, 1,1-diphenyl-2-picrylhydrazyl (DPPH), butylated hydroxyanisole (BHA), 2,6-di-t-butyl-1-hydroxytoluene (BHT), and trolox (Sigma Aldrich, Germany) was used to determine the antioxidant activity.

Table 1. Analytical parameters of the ICP-MS analysis method

Elements	Linear range (µg/kg)	y=mx+n	R^2	LOD (µg/kg)	LOQ (µg/kg)
Ca	50-2000	y=8.184x+493	0.9993	6.033	19.908
Na	25-2000	y=365.9x+34175	0.9998	2.947	9.725
Ba	25-200	y=279.2x-45.24	0.9997	0.130	0.430
K	10-2000	y=196.2x-11650	0.9999	0.043	0.141
Mg	10-2000	y=257.9x-334	0.9999	0.380	1.255
Fe	10-1000	y=22390x+289683	0.9998	0.407	1.343
Cr	5-1000	y=7016x-967	0.9999	0.119	0.393
Ti	5-1000	y=118x+91	0.9999	0.406	1.339
B	5-500	y=66.17x-4628	0.9998	0.078	0.258
Cu	5-500	y=1947x+3030	0.9999	0.561	1.852
Zn	5-500	y=2825x+10031	0.9998	0.914	3.015
Mn	5-500	y=30880x+5158	0.9999	0.059	0.193
Li	2.5-1000	y= 299.8x-529	0.9999	0.473	1.562
Co	2.5-1000	y=7083x+1067	0.9999	0.197	0.648
Be	2.5-500	y=41.36x+19.67	0.9998	0.338	1.118
V	2.5-500	y=113.6x+42.12	0.9999	0.132	0.435
Ni	2.5-500	y=24.35x+16.58	0.9999	0.303	1.001
As	2.5-500	y=10.36x+4.46	0.9998	0.141	0.465
Se	2.5-500	y=8.46x-1.16	0.9999	0.005	0.015
Mo	2.5-500	y=1496x+1949	0.9998	0.146	0.481
Cd	2.5-500	y=8482x+1397	0.9999	0.078	0.256
Sn	2.5-500	y=180.7x+53.84	0.9998	0.172	0.568
Al	1-500	y=12890x+124339	0.9999	0.301	0.992
Pb	1-100	y=4442x+17469	0.9998	0.269	0.888

2.3. Apparatus

Twenty-four element contents in the species samples were determined using the Bruker Aurora M90 model ICP-MS instrument (Table 2).

2.4. Preparation of the multi-element analysis of the species

Dry samples for multi-elemental analysis of *H. arenarium* species were ground in a mortar and brought homogeneously. Approximately 0.2150 g of the sample belonging to the G population and 0.2160 g from the B population were placed in the microwave teflon tubes. 10 mL of nitric acid was added to the tube and the solution mixture was stirred. For the pre-combustion process, the mixture in the tube was kept for 10 min. Subsequently, the tubes were closed and burned in the microwave oven.

For the burning program in the microwave oven: Initially, it was heated to 190 °C for 20 min, then was kept at 190 °C for 15 min (Pressure 800 psi. and power was set at 900-1800 watts) and finally was cooled from 190 °C to room temperature for 15 min. The mixture in teflon tubes was filtered with blue banded filter paper, taken into a 50 mL tube, and diluted up to 50 mL with ultrapure water.

2.5. Preparation of the species extracts

Approximately 200 mg of each species was placed in the tube and 5 mL of 75% (containing 0.1% formic acid) methanol was added to it and homogenized at 2000 rpm. The mixture was then kept in an ultrasonic water bath at 25 °C for 10 min and centrifuged at 2500 rpm for 10 min. Then, the supernatant in the tube was transferred to another tube and the extraction process was repeated 2 times as stated above and the resulting supernatants were made up to a final volume of 10 mL with methanol.

2.6. CUPRAC method

Cu^{+2} ion reduction capacity of the methanol extract of *H. arenarium* species, Apak et al. were made according to the method in 2007 [25]. 0.01 M 0.25 mL $CuCl_2$ solution was added to the falcon tube and $7.5x10^{-3}$ M 0.25 mL of methanolic Neocuproine solution was added to it and mixed. 1 M 0.25 mL of CH_3COONH_4 buffer was added to the mixture and mixed. Extracts or standards of *H. arenarium* species at different concentrations (20, 40, and 80 µg/mL) were added to the solution and incubated in the dark for 30 min. Then, the absorbance values of species samples, standards, and control groups at 450 nm were recorded. The increasing absorbance value

Table 2. ICP-MS instrument analytical conditions

Parameters	
RF Power (kW)	1.40
Plasma Gas Flow Rate (L/min)	15
Auxiliary Gas Flow Rate (L/min)	1.5
Sheath Gas Flow Rate (L/min)	0.11
Nebulizer Gas Flow Rate (L/min)	0.95
Read delay (sec.)	40
Gas	Argon
Purge Gas	Hydrogen
Repeat /Sample Reading	3
Scan replicate	5
Scan mode	Peak hopping
Hydrogen Gas Flow Rate (mL/min)	80

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of the reaction mixture indicates the Cu^{+2} ion reduction capacity.

2.7. DPPH method

DPPH free radical scavenging activity of the plant extracts was measured by the method described in Makhlouf-Gafsi et al. [26]. 0.1 mL of the plant extract was added to the tube. 3.9 mL of DPPH (in 60 µM-methanol) solution was added to it and mixed. The mixture and standards (0.125, 0.25, 0.50, 1.00 and 2.00 µg/mL) were incubated for 30 min and absorbance was read at 517 nm. The results were expressed in mg TE/1000g Trolox equivalent. All measurements were done in triplicate. The radical scavenging activity was calculated as a percentage of DPPH discoloration using the equation:

$$= \frac{\text{Acontrol} - \text{Asample}}{\text{Acontrol}} \; x\; 100$$

$A_{control}$ is the absorbance of the control reaction mixture, A_{sample} is the absorbance of the sample.

2.8. Statistical analysis

The analysis of the species was performed in three repetitions and the mean values of the data were used in the statistical analysis. The correlation between the metal contents of *H. arenarium* species belonging to different populations was determined using linear correlation with the SSPS 21 statistical package program (2012).

3. Results and Discussion

3.1. Multi-elemental analysis results of the species

K, Ca, Mg, Al, Fe, Na, Li, Be, B, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, As, Mo, Cd, Sn, Ba, and Pb element contents of the sample belonging to the B. population of the *H. arenarium* were detected as 251827, 5994, 1204, 395, 354, 60.53, 0.67, 0.13, 24.62, 59.64, 0.53, 5.95, 37.74, 0.24, 18.34, 3.58, 18.56, 0.01, 2.56, 0.19, 0.11, 2.54 and 0.62 mg/kg, respectively. In addition, Se metal content could not be detected in the sample belonging to the B population (Table 3).

K, Ca, Mg, Al, Fe, Na, Li, Be, B, Ti, V, Cr, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Cd, Sn, Ba and Pb element contents of the sample belonging to the G population of the *H. arenarium* species were detected as 281092, 5108, 1073, 314, 245, 49.92, 0.38, 0.11, 18.48, 52.15, 0.71, 3.34, 46.30, 0.35, 12.90, 6.30, 14.14, 0.05, 0.08, 1.04, 0.19, 0.19, 4.89 and 0.51 mg/kg, respectively (Table 3).

Very little attention has been paid to the content of macro-and microelements in *H. arenarium* species, herbal drugs, and extracts. To the best of our knowledge, only three reports were done and partly studied this subject. In a study by Lemberkovics et al. in 2002, the macro and microelement content of *H. arenarium* species has been determined using inflorescences and water extract. The element content of this species, which was grown in Hungary (Soroxar) in 2000 was determined as Al; 134.0±6.1, As; <1.0, B; <2.5, Ba; 8.1±2.0, Ca; 5309±46, Cd; <1.26, Co; <0.15, Cr; 3.1±0.5, Cu; 11.7±2.8, Fe; 31.1±9.7, Hg; <0.5 K; 15942±1587, Li; <0.15, Mg; 1661±62, Mn; 129.4±6.5, Mo; <0.05 Na; 46.3±7.6, P; 2830±198, Pb; <1.26, S; 2171±143, Ti; 1.6±0.4, V; <0.5 and Zn 28.0±1.2 mg/kg [27].

In the study to determine the gold and silver content of *H. arenarium* plant grown in Turkey, roots, stems, and flowers of the plant were used for Ag and Au contents. Ag and Au contents of the root part of the species were determined as 16.24 and 1.77 ppb (µg/kg), respectively. The element contents of the stem-leaves part were determined as 8.50 and 4.39 ppb (µg/kg), respectively. The element contents of the flower part were determined as 4.00 and 2.82 ppb (µg/kg), respectively [28].

In another study, the main and trace element contents of the root, leaf, and flower of *H. arenarium* have been investigated. The Ca, Mg, Na, Al, K, Fe, Mn, P, U, Sr, Rb, Ba, Ti, La, Ce, Zr, Cr, W, and Hg values in roots, stems, and flowers of the plant have been determined in the range of 0.46–1.4, 0.57–1.43, and 0.27–1.43%; 0.072–0.37, 0.1–0.286, and 0.098–0.147%; 0.001–0.04, 0.02–0.049, and 0.02–0.022%; 0.04–0.35 and 0.03–0.18%; 0.41–0.83, 0.97–2.24, and 1.37–1.98%; 0.065–0.5, 0.02–0.27, and 0.01–0.08%; 45–372, 34–227, and 21–83 (mg/kg); 0.062–0.13, 0.089–0.3, and 0.16–0.3 mg/kg; 0.01–0.15 and 0.001–0.1 mg/kg; 18–64.8, 17.3–64.8, and 4.8–19.3 mg/kg; 1–8.6, 1–11.5, and 2.4–14.5 mg/kg; 4.8–118.6, 4.2–116.8, and 0.9–24.9 mg/kg; 10–142, 4–86, and 3–16 mg/kg; 0.22–5.22, 0.13–3.7, and 0.02–1.09 mg/kg; 0.4–10.35, 0.2–7.13, and 0.05–2.33 mg/kg; 0.08–1.03, 0.08–0.8, and 0.02–0.38 mg/kg; 5.40–100.8, 3.1–88.2, and 2–13.1 mg/kg; 3.8–93.7, 2.6–

Table 3. ICP-MS results of examined species (mean concentration ± SD, n = 3)

Elements	B (mg/kg)	G (mg/kg)
K	25182±506	28192±969
Ca	5994±188	5108±54
Mg	1204±31	1073±2
Al	395±11	314±2
Fe	354±12	245±5
Na	60.53±2.45	49.92±1.05
Li	0.67±0.11	0.38±0.05
Be	0.13±0.03	0.11±0.00
B	24.62±2.26	18.48±0.87
Ti	59.64±2.21	52.15±1.56
V	0.53±0.08	0.71±0.14
Cr	5.95±0.14	3.34±0.20
Mn	37.74±1.34	46.30±1.08
Co	0.24±0.06	0.35±0.09
Ni	18.34±0.77	12.90±0.36
Cu	3.58±0.55	6.30±0.34
Zn	18.56±0.43	14.14±0.19
As	0.01±0.00	0.05±0.00
Se	N.D.	0.08±0.00
Mo	2.56±0.19	1.04±0.13
Cd	0.19±0.07	0.19±0.06
Sn	0.11±0.02	0.19±0.01
Ba	2.54±0.11	4.89±0.20
Pb	0.62±0.24	0.51±0.33

N.D.: Not detected < LOD

100, and 1.7–100 mg/kg and 2–14 and 1–12 µg/kg, respectively [29].

When the multi-element content of two different populations of the same species was compared, the element content of Ca, Mg, Al, Fe, Na, Li, Be, B, Ti, Cr, Ni, Zn, Mo, and Pb of the sample in the B population was determined that be higher than that of the other population. The element contents of K, V, Mn, Co, Cu, As, Se, Sn, and Ba of the sample in the G population were determined to be higher than in the sample from the other population (Table 3). The element content of the samples belonging to two different populations of the same species is different; it can be said that the species changes depending on genetic factors, geographical location, climatic factors, vegetation period, air pollution, and environmental factors.

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When the detected multi-elemental content of the species and the element contents found in the literature studies are evaluated, the macro, micro and toxic element contents were determined that be different from each other. As a result, the multi-element content of the species has been determined that vary depending on the geographical location of where the plant grows, climatic characteristics, air pollution, and environmental factors.

When the As, Cd, and Pb metal contents of *H. arenarium* species were compared with the maximum allowable concentration values in WHO raw plant materials; It was determined that the samples of the species belonging to two different populations were lower than the value determined by WHO. Thus, it can be said that the use of samples belonging to two different populations of this species in cosmetics, herbal medicine, and tea does not pose a health hazard.

3.2. Antioxidant results of the species

3.2.1. CUPRAC results

When the antioxidant activity results of *H. arenarium* species were evaluated; the Cu^{+2} reduction capacities of the sample extracts belonging to the B and G populations of the species were determined that were lower than the BHA, BHT and Trolox values used as standard at 20 and 40 µg/mL concentrations. It was determined that the Cu^{+2} reduction capacities of the sample extracts of both populations of the species showed activity close to the BHA, BHT, and Trolox used as standard at a concentration of 80 µg/mL (Figure 1, Table 4).

The Cu^{+2} reduction capacity of the sample extract belonging to the G population of the species was determined that be more active than BHA and BHT used as standard at a concentration of 80 µg/mL. When the sample extracts from two different populations of the same species were compared, the sample belonging to the G population was determined that show higher activity (Figure 1, Table 4).

Stankov et al. determined by the method of CUPRAC for antioxidant activity of 70% ethanol extract of H. arenarium (L.) Moench from Turkey and reported as 159.46 mM TE/g dw for the antioxidant value [30].

There are few available data regarding in determination of the antioxidant capacity by the CUPRAC method of *H. arenarium* species. In this case, a comparison with the results of other researchers is not possible. In contrast to this, there are various studies regarding in determination of the antioxidant capacity by the DPPH and ABTS method of *H. arenarium*.

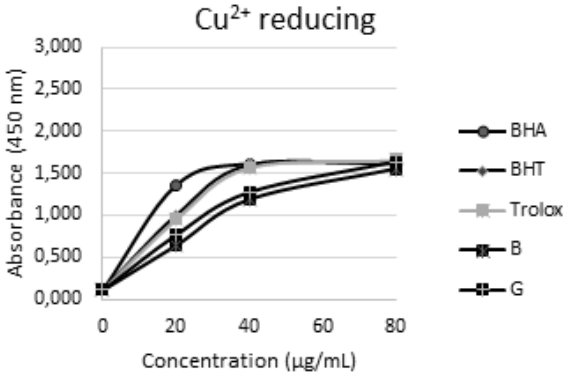

Figure1. Cu^{+2} reduction capacities of samples and standards from different population of *H. arenarium* species

3.2.2. DPPH results

DPPH radical scavenging method was used to evaluate free radical scavenging ability by *H. arenarium* extract belonging to different populations. The antioxidant activity of *H. arenarium* extracts tested by DPPH assay is presented in Table 4. The results obtained from the DPPH assay were presented as Trolox equivalents. The percentage of DPPH radical scavenging activity scavenged ranged from 3.85 to 36.60% (B) and 3.64 to 39.98% (G) for the *H. arenarium* extract (0.125 – 2.00 µg/mL) belonging to two different populations, respectively (Table 4). The obtained results indicated that investigated belonging to two populations samples act in a dose-dependent manner. A significant difference was not observed between the percentage of DPPH radical scavenging activity of the species belonging to different populations. However, the percentage of DPPH radical scavenging activity of the sample from population B was higher than that of population G at almost all concentrations. Also, the DPPH radical scavenging activity percentage of the extract belonging to the G population at a concentration of 2.00 µg/mL was determined that be higher than the other population (Table 4).

Previous data concerning the antioxidant activity of aqueous tea infusions of species were obtained by Kulisic-Bilusic et al. using the DPPH assay. These authors indicated that DPPH inhibition of aqueous extract of the *H. arenarium* in 0.5 g/L concentration possesses a value between 90 to 100% [31].

Kladar et al. (2015) has been reported the radical scavenging activity of the EtOH extract (0.25 – 1.25 µg/mL) of the *H. italicum* subsp. *italicum* using the DPPH assay that is ranged from 13.26 to 61.42% [12].

Kramberger et al. (2021) the radical scavenging activity of the infusions of the *H. arenarium* using the DPPH assay has been obtained as 4.4% [32].

A significant difference between the radical-scavenging potential of the B population extract (IC_{50} = 2.57 mg/mL) and the G population (IC_{50} = 2.48 mg/mL) was not noticed. However, the radical-scavenging potential of the B population extract was determined that be higher than the G population. When the DPPH antioxidant results of species extract from two different populations were evaluated, the B and G populations were determined as 22.95 and 23.76 mg TE/mL, respectively. Also, DPPH antioxidant results in 1000 grams of sample were determined as 1148 and 1188 mg TE/1000g, respectively (Table 4). The antioxidant results of the species belonging to both populations were determined that were close to each other. Thus, the antioxidant activity can be said that does not change in different populations of the same species from a regional perspective.

Babotă et al. (2018) has examined the antioxidant capacity using several extract methods for *H. arenarium*. The antioxidant results of the MeOH, EtOH, and EtOH 70% extracts of the species have been indicated as 4.91, 7.21, and 17.88 mg TE/mL, respectively [33]. In another study, the antioxidant result of *H. arenarium* leaf extract has been indicated as 19.13 mmol TE/L [34].

Table 4. Antioxidant activity results of the examined species (mean ± SD, n = 3)

CUPRAC					
Samples	20 (µg/mL)		40 (µg/mL)		80 (µg/mL)
B	0.642±0.035		1.190±0.031		1.554±0.056
G	0.766±0.043		1.273±0.026		1.637±0.063*
BHA	1.354±0.006		1.611±0.011		1.621±0.011
BHT	0.998±0.003		1.598±0.007		1.613±0.008
Trolox	0.953±0.024		1.573±0.015		1.661±0.050
DPPH					
DPPH radical scavenging activity (%)	**0.125 (µg/mL)**	**0.25 (µg/mL)**	**0.50 (µg/mL)**	**1.00 (µg/mL)**	**2.00 (µg/mL)**
B	3.85	6.78	16.21	24.02	37.60
G	3.64	6.97	11.48	20.32	39.98
Samples	**IC_{50} (µg/mL)**				
B	2.57				
G	2.48				
Trolox	0.059				
Samples	**DPPH Results (mg TE/mL)**		**DPPH Results (mg TE/1000g)**		
B	22.95±0.34		1148±38		
G	23.76±0.61		1188±62		

(*) was used to show that the antioxidant capacity at a concentration of 80 ug/mL of the species is higher than that of standards such as BHA and BHT.

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When the DPPH antioxidant results detected in this study and other studies in the literature are evaluated together, *H. arenarium* extract has determined that has antioxidant activity. The antioxidant activity results of *H. arenarium* belonging to different populations were determined that were close to each other and the antioxidant capacity of the species has been high. Also, the antioxidant capacity of the species in the literature has been reported that is high by using different extraction methods. Thus, the results determined and the results of the antioxidant in the literature can be said that are compatible with each other.

3.3. Bivariate Analysis

The Pearson correlation coefficient (r) is a measure of the linear relationship between two columns of data. Having a coefficient close to +1 or -1 of the r worth be indicating similarity between the two items (indicating positive and negative correlations). Having a value close to 0 indicates that the two items are weakly related or possibly unrelated.

According to the results of the bivariate analysis of the sample belonging to the B. population of the *H. arenarium* species, the Pearson correlation coefficient (r) at $p < 0.01$ confidence level, Na with Al ($r = -0.888$), Na with B ($r = -0.778$), Co with Zn ($r = -0.878$) reveals that there is a strong negative relationship between. When the Pearson correlation coefficients of the sample belonging to the B population of the species were compared, it was determined that the strongest correlation at $p < 0.01$ confidence level was between Na and Al. When the $P < 0.05$ confidence level of the Pearson correlation coefficient (r) of the sample belonging to the B population of the species is examined, V with Ba ($r = -0.763$), Na with Ti ($r = -0.709$), Na with Co ($r = -0.705$) and Fe and Cu ($r = -0.668$) reveals that there is a strong negative relationship. It reveals a strong positive correlation between Al with B ($r = 0.763$), Al with Ti ($r = 0.747$), B with Co ($r = 0.740$), and Mg with Cr ($r = 0.648$). When the Pearson correlation coefficients of the sample belonging to the B population of the species were compared, at a confidence level of $p < 0.05$ it was determined that the strongest correlation was between Al and B and between V and Ba (Table 5). In addition, when the $p < 0.01$ and 0.05 confidence levels of the sample belonging to the B population of the species were detected, the strongest correlation was found with Na and Al. A significant correlation between two or more elements indicates a similar ability or presence of the same source for *H. arenarium* species. The dependence between the elements with the strongest correlation above can be explained by the common origin of the metals.

According to the results of the bivariate analysis of the sample belonging to the G population of the *H. arenarium* species, the at $p < 0.01$ confidence level reveals that there is a strong positive correlation between Al with B ($r = 0.844$) and between Al with Ti ($r = 0.841$). It reveals that there is a strong negative relationship between Ca with Mn ($r = -0.827$). When the Pearson correlation coefficients of the sample belonging to the G population of the species were compared, at a confidence level of $p < 0.01$, it was determined that the strongest correlation was between Al with B. When the $P < 0.05$ confidence level of the Pearson correlation coefficient (r) of the sample belonging to the G population of the species is examined, it is revealed that there is a strong positive correlation between Fe with B ($r = 0.636$) and Na with Cr ($r = 0.632$). When the Pearson correlation coefficients of the sample belonging to the G. population of the species were compared with a confidence level of $p < 0.05$, it was determined that the strongest correlation was between Fe and B (Table 6). In addition, when the $P < 0.01$ and 0.05 confidence levels of the sample belonging to the G population of the species were examined, it was determined that the strongest correlation was between Al and B.

By looking at the correlation coefficients of the samples belonging to two different populations of the same species, information that can show only the same source or behavior for two or more items can be obtained, while information about the differences between samples taken from different regions cannot be obtained.

4. Conclusions

Multi-elemental contents of *H. arenarium* species belonging to different populations were analyzed and compared. It was determined that the metal contents of two different populations of the species were different from each other and some element contents were higher than the other population. Thus, it has been determined that the multi-elemental content of the samples from different populations will vary depending on geographical location, climatic factors, air pollution, and environmental factors.

Table 5. Correlation matrix of metal concentrations of the sample belonging to the B population of *H. arenarium* species

	K	Na	Mg	Ca	Al	Fe	Li	B	Ti	V	Cr	Mn	Co	Ni	Cu	Zn	Mo	Ba	Pb
K	1																		
Na	0.123	1																	
Mg	-0.209	0.067	1																
Ca	0.040	0.020	-0.230	1															
Al	0.158	-0.888**	-0.069	-0.236	1														
Fe	0.028	-0.284	-0.152	0.233	0.297	1													
Li	0.094	-0.453	-0.479	0.282	0.333	0.412	1												
B	0.254	-0.778**	-0.049	0.122	0.763*	0.125	0.623	1											
Ti	0.342	-0.709*	0.087	-0.126	0.747*	0.533	0.295	0.598	1										
V	0.233	0.263	0.264	0.364	-0.372	-0.376	0.217	0.138	-0.246	1									
Cr	-0.045	-0.163	0.648*	-0.550	0.219	-0.344	-0.109	0.371	0.226	0.141	1								
Mn	-0.017	0.065	-0.007	-0.093	-0.219	-0.352	-0.016	0.113	-0.003	-0.003	0.467	1							
Co	-0.128	-0.705*	0.030	0.414	0.594	0.027	0.390	0.740*	0.260	0.191	0.060	-0.264	1						
Ni	-0.196	-0.387	0.586	0.073	0.377	0.212	-0.386	0.118	0.434	-0.169	0.150	-0.355	0.448	1					
Cu	0.358	-0.157	-0.007	-0.554	0.321	-0.668*	-0.012	0.336	0.077	0.223	0.493	0.159	0.115	-0.178	1				
Zn	0.124	0.558	-0.257	-0.236	-0.502	0.158	-0.052	-0.484	-0.167	-0.265	-0.055	0.484	-0.878**	-0.612	-0.239	1			
Mo	0.006	0.055	0.324	-0.319	0.120	0.130	-0.515	-0.078	0.232	-0.608	0.409	0.354	-0.255	0.349	-0.142	0.316	1		
Ba	-0.389	-0.040	-0.273	-0.427	0.030	0.295	0.016	-0.231	0.082	-0.763*	0.063	0.357	-0.423	-0.176	-0.204	0.611	0.498	1	
Pb	0.370	0.238	0.017	-0.131	-0.132	0.005	0.225	-0.125	0.009	0.560	-0.119	-0.493	-0.154	-0.172	0.297	-0.067	-0.627	-0.415	1

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Table 6. Correlation matrix of metal concentrations of the sample belonging to the G population of *H. arenarium* species

	K	Na	Mg	Ca	Al	Fe	Li	B	Ti	V	Cr	Mn	Co	Ni	Cu	Zn	Mo	Ba	Pb
K	1																		
Na	0.162	1																	
Mg	0.310	0.361	1																
Ca	0.502	0.273	0.314	1															
Al	0.391	-0.237	0.523	0.499	1														
Fe	-0.204	0.107	0.026	0.339	0.4	1													
Li	-0.483	0.552	0.078	-0.340	-0.504	0.078	1												
B	0.374	-0.274	0.176	0.496	0.844**	0.636*	-0.57	1											
Ti	0.105	-0.52	0.204	0.287	0.841**	0.254	-0.487	0.616	1										
V	0.343	0.32	0.031	-0.026	-0.381	-0.620	-0.187	-0.449	-0.400	1									
Cr	-0.293	0.632*	0.594	0.208	0.042	0.227	0.494	-0.208	-0.116	-0.108	1								
Mn	-0.368	0.005	-0.138	-0.827**	-0.434	-0.169	0.444	-0.429	-0.294	0.223	-0.179	1							
Co	-0.054	-0.604	0.071	-0.041	0.105	-0.573	-0.496	-0.158	0.321	0.138	-0.095	-0.284	1						
Ni	0.068	-0.048	0.400	-0.029	0.448	0.423	-0.175	0.574	0.136	-0.079	-0.044	0.269	-0.268	1					
Cu	-0.128	-0.278	-0.171	-0.212	-0.450	-0.376	-0.159	-0.260	-0.528	0.214	-0.215	-0.012	0.430	0.077	1				
Zn	-0.034	0.461	0.003	0.404	-0.102	0.281	0.553	-0.174	-0.024	-0.288	0.347	-0.249	-0.400	-0.533	-0.502	1			
Mo	0.372	-0.158	0.294	0.389	0.194	-0.477	-0.115	-0.079	0.223	-0.036	-0.04	-0.513	0.535	-0.405	0.141	0.27	1		
Ba	-0.554	-0.349	0.218	-0.340	0.088	-0.025	0.248	-0.091	0.250	-0.220	0.051	0.415	0.257	0.376	0.146	-0.186	0.108	1	
Pb	-0.112	0.128	0.359	0.269	0.422	0.238	-0.296	0.226	0.435	0.252	0.413	-0.072	0.139	0.311	-0.352	-0.206	-0.318	0.143	1

* Correlation is significant at the 0.05 level. ** Correlation is significant at the 0.01 level.

The Cu^{+2} reducing capacities of *H. arenarium* species at 20 and 40 µg/mL concentrations have been lower than that of standard compounds BHA, BHT, and Trolox. Thus, the difference between samples at these concentrations became clear. The Cu^{+2} reduction capacity of the species sample at 80 µg/mL concentrations has determined that be close to the standards. In addition, the Cu^{+2} reduction capacity of the species sample at 80 µg/mL concentration determined that be higher than the BHA and BHT standards.

The CUPRAC and DPPH antioxidant results of the species belonging to different populations have been determined that be close to each other. It has been determined that the species had an antioxidant activity with both methods and there is no regional difference in the activity results. Consequently, *H. arenarium* reveals that the species has antioxidant activity and its usability in the field of health.

According to the results of the bivariate analysis of the species, information on the differences between the samples belonging to different populations could not be obtained. Thus, it has been determined that more complex analysis systems should be used to determine the difference from a regional angle.

Conflict of Interest

The authors declare no competing financial interest.

Statement of Contribution of Researchers

Concept – A.U., K.U., F.A.; Design – A.U., K.U.; Supervision – I.A., F.A.; Resources – A.U., K.U.; Materials – A.U., K.U.; Data Collection and/or Processing – A.U., K.U.; Analysis and/or Interpretation – A.U., K.U., F.A., I.A.; Literature Search – K.U., A.U., I.A.; Writing – A.U.; Critical Reviews – F.A., I.A.

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