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The Investigation on Antioxidant Activities of *Nasturtium Officinale* Extracts and Its Mineral Content

Nasturtium Officinale Ekstraktlarının Antioksidan Aktiviteleri ve Mineral İçeriğinin Araştırılması

Leyla ERCAN [1,*], Mehmet DOĞRU [2]

[1] *Dicle University, Institute of Science, 21280, Diyarbakır, Turkey*
[2] *Dicle University, Faculty of Ziya Gökalp Education, Department of Chemistry, 21280, Diyarbakır, Turkey*

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ABSTRACT

Antioxidants prevent many diseases by eliminating the harmful effects of free radicals. In this study, it was aimed to determine the antioxidant capacity of *Nasturtium officinale* extracts prepared by different methods, as well as to determine the Ca, Mg, Zn, Fe, K minerals. Antioxidant activities of methanol and water extracts of *Nasturtium officinale* were determined by different in vitro methods such as DPPH, ABTS, DMPD, CUPRAC, FRAP, and Fe^{3+} Fe^{2+} reduction assays. The results were compared with the standard antioxidants (BHA, BHT, and Trolox). Both water and methanol extracts presented high antioxidant potential on ABTS free radical scavenging assay. While water extract showed better antioxidant properties in the DMPD experiment, methanol extract showed higher antioxidant properties than water extract in other methods. In addition to the chlorophyll, carotenoid, total phenolic, and total flavonoid analyzes of *Nasturtium officinale,* beneficial minerals of the plant were also revealed in ICP-MS. We determined that *Nasturtium officinale* is an important herb with a good source of minerals.

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ÖZ

Antioksidanlar, serbest radikallerin zararlı etkilerini yok ederek birçok hastalığı önler. Bu çalışmada, farklı yöntemlerle hazırlanan *Nasturtium officinale* ekstraktlarının antioksidan kapasitesinin belirlenmesinin yanı sıra Ca, Mg, Zn, Fe, K mineralleri içeriğinin belirlenmesi amaçlanmıştır. *Nasturtium officinale*'nin metanol ve su ekstraktlarının antioksidan aktiviteleri DPPH, ABTS, DMPD, CUPRAC, FRAP ve Fe^{3+} Fe^{2+} indirgeme deneyleri gibi farklı in vitro yöntemlerle belirlendi. Sonuçlar, standart antioksidanlar (BHA, BHT ve Trolox) ile karşılaştırıldı. Hem su hem de metanol ekstraktları, ABTS serbest radikal süpürme tahlilinde yüksek antioksidan potansiyel gösterdi. DMPD deneyinde su ekstraktı

***Corresponding Author**

E-mail addresses: leylaercan@artuklu.edu.tr (Leyla ERCAN), mehmetdogru21@gmail.com (Mehmet DOĞRU)

daha iyi antioksidan özellik gösterirken, metanol ekstraktı diğer yöntemlerde su ekstraktından daha yüksek antioksidan özellik göstermiştir. Ayrıca *Nasturtium officinale*'nin klorofil, karotenoid, toplam fenolik ve toplam flavonoid analizlerinin yanı sıra ICP-MS cihazında bitkinin faydalı mineralleri de ortaya çıkarılmıştır. *Nasturtium officinale*'nin iyi bir mineral kaynağı olan önemli bir bitki olduğunu tespit ettik.

1. INTRODUCTION

Nasturtium officinale, a member of the Brassicaceae family, is an aquatic plant [1]. *Nasturtium officinale* usually grows in freshwaters, marshes, and wetlands. Plants are natural sources that contain many compounds and minerals that the human body needs. Some of these components show antioxidant properties. Antioxidants protect from many diseases and damage by balancing the free radicals produced during metabolic processes [2]. Dietary natural antioxidants reduce the possibility of diseases such as cancer, cardiovascular diseases, and cataracts [3]. In cases where antioxidant defense systems are insufficient against free radicals, dietary intake of natural antioxidants is required. Therefore, it becomes important to determine the antioxidant capacity of foods and plants. Due to the different chemical properties of antioxidants, different methods have been developed to determine antioxidant capacity. Among these methods, studies are carried out to find the most efficient method, the cheapest, and easily reproducible according to the sample, and the methods are constantly renewed by trying different methods. For this purpose, the antioxidant capacity of *Nasturtium officinale* was determined by using different methods and solutions in this study.

The macrominerals K, Ca, Mg and the microminerals Fe and Zn are involved in various metabolic processes and are important for both humans and other living things. Ca, which is necessary for humans, is involved in many metabolic events such as bone and dental health, the functioning of muscle and nerve tissues, communication between the brain and the body, blood circulation, and the hormonal system [4]. Fe, which is an important mineral for the human body and other living things, is responsible for the energy metabolism in the organism. It takes part in oxygen transport, electron transfer, redox reactions. Iron deficiency negatively affects reproductive health, growth, and cognitive development reduces work capacity, and causes economic losses. It may also cause anemia and mental disorders in pregnant women and children [4-5]. Previous studies have shown that high heme iron intake leads to increased body iron stores and is significantly associated with the risk of developing type 2 diabetes mellitus [6]. However, dietary intake of non-heme iron and iron supplements is not associated with type 2 diabetes [7]. Potassium (K), which is a cofactor for many enzymes, is the most important cation in the cell. High K concentration is necessary for both cell metabolism, cell division, acid-base balance as well as O_2 and CO_2 transport, energy transfer, protein, and carbohydrate metabolism [4]. Mg is required for all ATP-utilizing enzymes. Mg also functions as an allosteric activator of enzymes,

including critical enzyme systems such as adenylate cyclase, phospholipase, and Na/K-ATPase [8-9]. Symptoms of Mg deficiency include vertigo, muscle tremors, and fatigue. One study found that a diet that increased Mg intake significantly lowered blood pressure [10]. Zinc is an important trace element for living things, which acts as the active center of many enzymes. Zn also acts as a cofactor for enzymes such as carboxyl peptidase, alcohol dehydrogenase, and carbonic anhydrase [11]. Decreased sense of taste, decreased sperm count, growth retardation, skin abnormalities, anorexia, lethargy, delayed wound healing, dermatitis, diarrhea, baldness, and mental abnormalities may occur in zinc deficiency [12]. In addition to inadequate and wrong nutrition, diseases caused by mineral deficiency can be seen during pregnancy and childhood when the need for these minerals increases. For example, as a result of iron deficiency, weakness, fatigue, decreased cognitive efficiency and difficulty concentrating may be experienced due to low oxygen delivery to body tissues [13]. In addition, a relationship has been suggested between low levels of Zn, Fe, Mg and the occurrence of depressive symptoms in postmenopausal women [14]. Considering the many diseases caused by mineral deficiency and the economic losses caused by these diseases, it is important to determine the mineral content of foods.

It is important to reveal the antioxidant capacity and minerals in plants to find out their valuable aspects. Therefore, in this study, we examined the antioxidant activities of *Nasturtium officinale*, the amount of minerals in terms of Ca, Mg, K, Fe, Zn.

2. MATERIAL AND METHOD

2.1 Plant Material and Chemicals

The plants used in our study were collected from the natural environment in Kayseri, Turkey. Dried (watercress) *Nasturtium officinale* was used by grinding. The plant samples used in our study were authenticated by Prof. Dr. Hasan AKAN.

Chemicals; ABTS (2, 2-azinobis 3-ethylbenzthiazoline-6-sulfonic acid), trolox (6-hydroxy-2,5,7,8-tetramethyl chromane-2-carboxylic acid)), BHT (butylated hydroxytoluene-supelco), BHA (butylated hydroxyanisole), DPPH (1,1-diphenyl-2-picryl-hydrazyl), TPTZ (2,4,6-tris (2-pyridyl)-s-triazine), neocuproine, DMPD (N,N-Dimethyl-p-phenylenediamine dihydrochloride), TCA (Trichloroacetic acid-supelco) and $CuCI_2$ were purchased from Sigma-Aldrich (Germany).

2.2 The Determination of Chlorophyll and Carotenoid Amount

0.1 g of fresh plant sample was weighed and 10 ml of acetone (80%) was added and homogenized. The extract (acetone part) was taken and the extraction process was repeated (2 times)

until colorlessness was obtained and the extracts were collected in a tube. The mixture was filtered and the volume made up to 25 ml with acetone. Absorbances were recorded at 663 nm, 652 nm, 645 nm, and 470 nm [15-16].

Chlorophyll amounts were calculated according to the formula below;

$$\text{Chlorophyll}_a = (12.7 \times A_{663} - 2.7 \times A_{645})(v / (1000 \times w)$$

$$\text{Chlorophyll}_b = (22.9 \times A_{645} - 4.68 \times A_{663})(v / (1000 \times w)$$

$$\text{Total Chlorophyll} = (27.8 \times A_{652})(v / (1000 \times w)$$

$$\text{Total Carotenoid} = (1000 \times A_{470} - 1.63 \times \text{Chlorophyll}_a \times 104.96 \times \text{Chlorophyll}_b) / 221$$

$(v / (1000 \times w)) =$ dilution factor, w : the amount of sample weighed (g),
v : Final volume of extract prepared with 80% acetone (ml).

2.3 Preparation of Samples for Determination of Total Phenolic Amount, Total Flavonoid Amount, and Antioxidant Capacity

To determine the total phenolic, total flavonoid, and antioxidant capacity, two different extracts of the plant (water and methanol) were prepared. The collected plant samples were dried in the shade and crushed with a shredder. To investigate the antioxidant capacity of both hydrophobic and hydrophilic content of the plant, two separate extractions were prepared in distilled water and methanol. To prepare the lyophilized extract, 200 ml of distilled water was added to 30 grams of plant sample and 200 ml of methanol was added to 30 grams of plant sample to prepare the evaporated sample. It was then stirred at room temperature for 12 hours with a magnetic stirrer. The solutions were then filtered through a filter paper. The prepared aqueous solution was frozen in a deep freezer. The frozen extract was lyophilized in a lyophilizer under the pressure of 50 mm-Hg. The methanol solution was evaporated at a pressure of 150 millibars and 35°C. To prepare a stock solution from lyophilized and evaporated extracts, 20 ml solvent (water and methanol) was added in 20 mg extract.

2.4 The Determination of Total Amount of Phenolic

Total phenolic content was determined using the Folin-Ciocalteu reagent [17-18]. The results were expressed in mg of gallic acid equivalent gallic acid (GAE) per g weight sample. For this 100 µl extracts were taken into a tube. 900 µl of distilled water and 5 ml of FCR (Folin Ciocalteu reagent 10%) were added and mixed in a vortex. 5 ml of sodium carbonate (7.5%) was added and mixed in the vortex for 20 seconds. This mixture was kept in the dark for 2 hours at room temperature. The absorbances were recorded in the spectrophotometer at 765 nm.

2.5 The Determination of Total Amount of Flavonoids

0.4 ml of extract was taken to determine the total amount of flavonoids [19-20]. 4 ml of distilled water and 0.3 ml of $NaNO_2$ (5%) were added. After 5 minutes, 0.5 ml of $AlCl_3$ (10%) was added and mixed. After 6 minutes, 2 ml of NaOH (1 M) and 3 ml of distilled water were added. The mixture was mixed and absorbance was recorded at 510 nm. The results were determined as mg equivalent catechin in the g sample.

2.6 Antioxidant Capacity Analysis

To the determination of DPPH• (1,1-diphenyl-2-picryl-hydrazyl) radical scavenging activity of water and methanol extracts of *Nasturtium officinale*, 250 µM DPPH• solution was prepared [21]. Extracts at different concentrations were taken and made up to 3 ml with methanol and 1 ml of DPPH• was added. Absorbance was recorded at 517 nm after 30 minutes in the dark. As control solution 1 ml DPPH • solution and 3 ml methanol was used. Antioxidant concentrations (IC_{50}) causing 50% inhibition were calculated.

To determine the radical scavenging activity of ABTS• (2,2-Azinobis-3-ethylbenzothiazoline-6-sulfonic acid), ABTS radical was formed by mixing 50 ml of 2 mM ABTS with 25 ml of 2.45 mM potassium persulfate [22-23]. Before using the $ABTS^{+\bullet}$ radical solution, its absorbance at 734 nm was adjusted to a value in the range 0.750 nm - 0.800 nm with 0.1 M and pH 7.4 phosphate buffer. The stock solutions at different concentrations were taken into test tubes and 2 ml of ABTS radical solution was added and the total volume was completed to 3 ml with phosphate buffer. After 30 minutes of incubation, absorbances were recorded at 734 nm.

To determine the DMPD radical scavenging activity, 0.4 ml of 0.05 M $FeCl_3$ and 2 ml of 0.1 M DMPD solution were added to 100 mL of acetate buffer (pH: 5.3; 0.1 M) to obtain radical cation ($DMPD^{\bullet+}$) [24]. Before using the DMPD+ radical solution, its absorbance was adjusted to 0.900±0.100 at 505 nm. Solutions prepared at different concentrations were transferred to test tubes. Then, 1 ml of $DMPD^{\bullet+}$ solution was added to them. After 50 minutes of incubation, absorbances were measured at 505 nm.

To determine the copper ion ($Cu^{2+)}$ reducing capacity, 0.01 M $CuCl_2$ solution, $7.5x10^{-3}$ M methanolic neocuprin solution, and 1 M ammonium acetate buffer (pH= 6.5) were added to the samples taken at different concentrations [25]. After 30 minutes of incubation, absorbance at 450 nm was recorded.

The volumes of the samples taken at different concentrations for the determination of antioxidant capacity by FRAP method were completed to 0.5 ml with buffer solution (pH=3.6 acetate buffer) [26]. Then, 20 mM $FeCl_3$ solution and FRAP reagent (10 volumes of 0.3 M acetate buffer + 1 volume of 10mM TPTZ solution + 1 volume of 20mM $FeCl_3.6H_2O$ solution) were added to the test tubes. After 10 minutes, absorbances were recorded at 593 nm.

To determine the Fe^{3+} Fe^{2+} reducing capacity, 1 ml 0.2 M phosphate buffer (pH: 6.6) and 1 ml 1% potassium ferricyanide $K_3Fe(CN)_6$ were added to the samples prepared at different concentrations. After incubation at 50°C for 20 minutes, 1 ml of 10% TCA and 0.25 ml of 0.1% $FeCl_3$ were added and absorbance was recorded at 700 nm [27].

2.7 The Determination of Mineral Content Using ICP-MS

Inductively coupled plasma mass spectrometry (ICP-MS) for mineral analysis in the Bruker aurora M90 model, 0.2 g of plant sample was placed in a microwave tube. 10 ml of nitric acid was added to it. The solution mixture was mixed and pre-burning was carried out in 10 minutes. Later, the tubes were covered and fired in a microwave oven. The firing program in the microwave oven was respectively carried out as follows. Initially, the microwave was heated to 190℃ for 20 minutes. Then it was kept at 190℃ for 15 minutes. The pressure was set at 800 psi, power at 900-1800 watts. Then it was cooled down from 190℃ to room temperature in 15 minutes [28]. Samples taken from the tubes after burning were completed up to 50 ml with ultrapure water. Analysis was performed on the Bruker brand aurora M90 model ICP-MS device. Argon gas was used as plasma gas and Hydrogen gas was used as the purge gas. The hydrogen gas flow rate (milliliters /minute) was set at 80. It was analyzed on ten standard and blank devices. Calibrations plotted. Then, samples were analyzed.

3. THE RESEARCH FINDINGS

3.1 Total Phenolic, Total Flavonoid, Total Chlorophyll, Total Carotenoid and Antioxidant Capacity Results

Total phenolic and total flavonoid amounts of *Nasturtium officinale* are given in Table 1. The methanol extract is more efficient in phenolic and flavonoid determination analysis than the water extract.

Chlorophyll and carotenoid amounts of *Nasturtium officinale* are given in Table 2 The amount of carotenoid, which has many health benefits, has been determined.

Table 1. Total phenolic, total flavonoid amounts of *Nasturtium officinale*

Analysis	Water Extract	Methanol Extract
Total phenolic content (equivalent gallic acid mg/g)	148.5± 10 mg GAE/g sample	211.5±6 mg GAE/g sample
Total flavonoid amount (equivalent catechin mg /g)	13.5±3 mg catechin /g sample	81.83± 8 mg catechin /g sample

Table 2. Chlorophyll and carotenoid amounts of *Nasturtium officinale*

Analysis	*Nasturtium officinale*
Chlorophyll a	2.24 ± 0.01 mg/g
Chlorophyll b	2.25 ± 0.3 mg/g
Total chlorophyll	4.47 ± 0.2 mg/g
Total carotenoid	1.15 ± 0.3 mg/g.

According to Table 2, in the *Nasturtium officinale* is chlorophyll a = 2.24 ± 0.01 mg/g, chlorophyll b = 2.25 ± 0.3 mg/g, total chlorophyll = 4.47 ± 0.2 mg/g. Total carotenoid in *Nasturtium officinale* is 1.15 ± 0.3 mg/g.

The DPPH free radical scavenging activity of *Nasturtium officinale* extracts and standard antioxidants (BHA, BHT, Trolox) was investigated. According to the results, it was found that the methanol extract of the plant has effective DPPH radical scavenging activity but less than standard antioxidants. DPPH free radical inhibition percentages of the extracts and standard antioxidants at the same concentration (40 μg/ml) were determined as Trolox (90.97%) >BHA (83.65%) > BHT (31.53%) > methanol extract (16.56%) > water extract (6.05%). In addition, the IC_{50} values of the methanol and water extract of the plant and the standard antioxidants (concentration of a sample to scavenge 50 % of free radicals) are given in Table 3. ABTS cation radical inhibition percentages of extracts and standard antioxidants at the same concentration (40 μg/ml) decreased in the order of Trolox (99.53%)> BHA (99.44%) > BHT (78.87%) > methanol extract (41.55%) > water extract (26.06%). The IC_{50} values of the extracts and standards are given in Table 3. DMPD radical inhibition percentages of extracts and standard antioxidants at the same concentration (40 μg/ml) decreased in the order of Trolox (69.29%) >BHA (26.30%) > water extract (20.98%) > methanol extract (11.36%). The IC_{50} values of the extracts and standards are given in Table 3.

Table 3. Radical scavenging IC_{50} values of standard antioxidants and *Nasturtium officinale*

Standard Antioxidants and Extracts	DPPH Radical Scavenging	ABTS Radical Scavenging	DMPD Radical Scavenging
BHA	23.07±0.3 μg/ml	13.7±0.1 μg/ml	83.68±6 μg/ml
Trolox	18.79±0.2 μg/ml	18.1±0.1 μg/ml	18.14±2 μg/ml
BHT	63.78±0.1 μg/ml	26.79±0.5 μg/ml	-
Methanol extract	120.92±0.7 μg/ml	46.54±0.3 μg/ml	220.98±30 μg/ml
Water extract	320.16±1.9 μg/ml	92.03±1.5 μg/ml	97.60±6 μg/ml

According to Table 3, it is seen that the methanol extract is more efficient than the water extract in the analysis of antioxidant capacity determination.

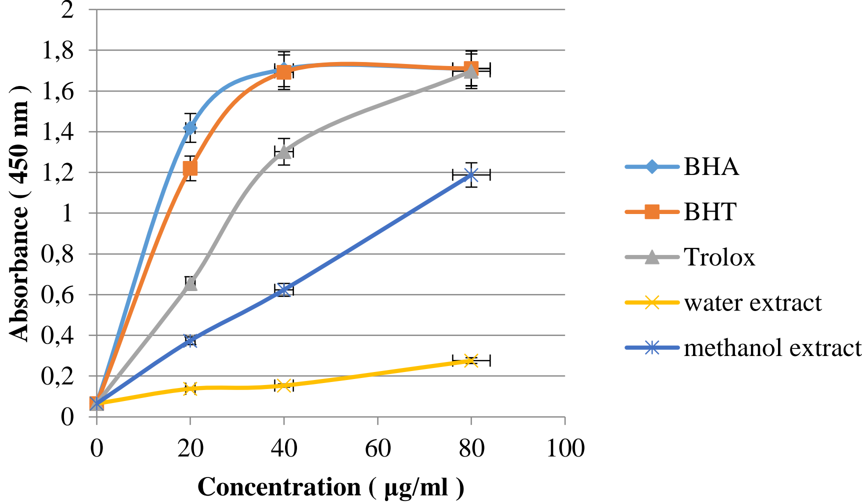

Figure 1. *Reducing powers of Nasturtium officinale extracts and standard antioxidants by CUPRAC method*

The copper ions (Cu^{2+}) reduction potential of *Nasturtium officinale* extracts was experimentally determined and compared with BHA, BHT, and Trolox. At the same concentration (40 µg/ml), the cupric ions reduction potential of extracts and standard antioxidants decreased in the order of BHA>BHT>trolox>methanol extract>water extract (Figure 1).

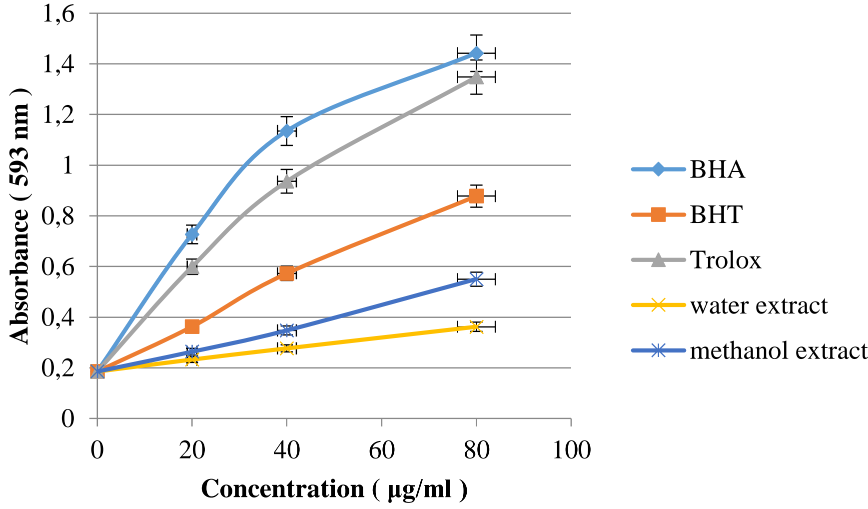

Figure 2. *Reducing powers of Nasturtium officinale extracts and standard antioxidants by FRAP method*

The FRAP reducing capacity of *Nasturtium officinale* extracts was determined and compared with BHA, BHT, and Trolox. At the same concentration (40 µg/ml), the FRAP reduction capacities of standard antioxidants and extracts were BHA> trolox> BHT>methanol extract>water extract, respectively (Figure 2).

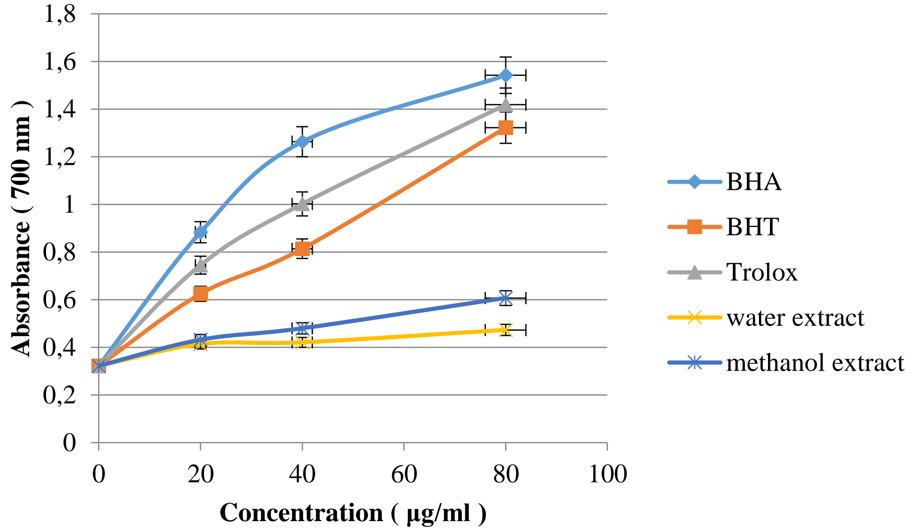

Figure 3. *Reducing powers of Nasturtium officinale extracts and standard antioxidants by Fe^{3+} Fe^{2+} reducing capacity*

The Fe^{3+} Fe^{2+} reducing capacity of *Nasturtium officinale* extracts was determined experimentally and compared with BHA, BHT, and Trolox. At the same concentration (40 µg/ml), the Fe^{3+} Fe^{2+} reducing capacities of standard antioxidants and extracts were BHA> trolox> BHT>methanol extract>water extract, respectively (Figure 3).

Both extracts of *Nasturtium officinale* gave better results, especially in the ABTS assay. In five methods (DPPH, ABTS, CUPRAC, FRAP, Fe^{3+} Fe^{2+} Reducing), methanol extract gave more efficient results than water extract. But water extract gave more efficient in DMPD radical scavenging method. Considering the results, the antioxidant property of *Nasturtium officinale* has been shown comparatively. The results revealed that *Nasturtium officinale* has radical scavenging activity and antioxidant abilities.

3.2 ICP-MS Results

According to the analysis results of *Nasturtium officinale* given in Table 4, the plant 1554.71 ± 1.57 mg/kg Mg, 12628.97 ± 13.34 mg/kg K, 2023.36 ± 9.87 mg/kg Ca, 4387.92 ± 60.4 mg/kg Fe and 629.1 ± 0.93 mg/kg Zn contains.

Table 4. Analysis results of Nasturtium officinale in terms of Mg, K, Ca, Fe, Zn minerals

Minerals	ppm (mg/kg)	R^2	LOD (mg/L)	LOQ (mg/L)
Mg	1554.71±1.57	0.9999	0.38019	1.254628
K	12628.97±13.34	0.9999	0.042626	0.140664
Ca	2023.36±9.87	0.9993	6.032868	19.90847
Fe	4387.92±60.4	0.9998	0.407001	1.343103
Zn	629.1±0.93	0.9998	0.913527	3.014639

Significance: LOD: Limit of Detection, LOQ: Limit of Quantitation R^2: Correlation Coefficient

According to the mineral analysis results given in Table 4, *Nasturtium officinale* contains 1554.71 mg/kg Mg, 12628.97 mg/kg K, 2023.36 mg/kg Ca, 4387.92 mg/kg Fe and 629.1 mg/kg Zn. *Nasturtium officinale* plant is an excellent plant to meet the mineral needs compared to the amounts (375 mg for Mg, 2000 mg for K, 800 mg for Ca, 14 mg for Fe, 10 mg for Zn) which the Turkish Food Codex has determined as daily reference intake values for vitamins and minerals [29]. It has been demonstrated that the plant is a good source of minerals. The mineral content of the plant may vary depending on the environment in which it grows.

4. CONCLUSION

In this study, the antioxidant capacity of two different extracts of *Nasturtium officinale* was investigated from different aspects with six different antioxidant methods, and better results were observed in ABTS and CUPRAC methods. The methanol extract was showed better results compared to water extract. Components that dissolve in methanol and are insoluble in water may have been effective in this. Additionally, the mineral content of the plant, total phenolic amount, total flavonoid amount, and chlorophyll amount were determined and contributed to the literature. It has been proven that *Nasturtium officinale* is a species that can be the subject of much researches in medicine with the valuable components it contains. The benefits of the *Nasturtium officinale* that is collected from its natural environment and not noticed by people are pointed out.

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CONFLICTS OF INTEREST

No conflict of interest was declared by the authors.

AUTHORS' CONTRIBUTIONS

Leyla ERCAN: Writing-original draft preparation, data collection, data curation, visualization, analysis, data interpretation. Mehmet DOĞRU: Conceptualization, methodology, validation, writing-review, and editing, supervision, provision of analysis tools. All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

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