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Evaluation of Nutritional Value and Phytochemical Properties of Spinach (*Spinacia oleraceae*L.) in Different Habitats of Khuzestan Province

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ABSTRACT

Abstract

This study investigated the photosynthetic pigments and chemical compositions in spinach samples collected from across Khuzestan province. The results revealed significant differences in the levels of chlorophylls and other pigments. The highest amount of chlorophyll a was found in Hamidieh and Shadegan, while the lowest levels were measured in Abadan, Dezful, and Ramhormoz. Similarly, the highest chlorophyll b content was observed in Hamidieh, with the lowest amounts recorded in Ramhormoz, Abadan, and Dezful. Carotenoid analysis showed that the lowest concentration was in Hamidieh, while the highest levels were found in other habitats. In terms of anthocyanins, the highest levels were seen in Ramhormoz, while the lowest were in Abadan and Dezful. Regarding chemical compositions, the highest carbohydrate content was found in Hamidieh and the lowest in Ramhormoz. The total protein content was lowest in Shadegan. Additionally, significant differences were observed in the levels of various vitamins and mineral elements. Vitamin C levels were highest in Abadan, vitamin E in Dezful and Hamidieh, vitamin B_{12} in Ramhormoz and Shush, and folic acid in Ramhormoz. As for mineral elements, the highest calcium levels were observed in Dezful, copper in Shush, iron in Dezful, and magnesium in Ramhormoz. Furthermore, levels of heavy metals such as zinc, cesium, and lead varied significantly across the samples. The antioxidant capacity of spinach from Shadegan was the lowest, while the electrical conductivity of spinach from Ramhormoz was the highest. These findings highlight substantial differences in the chemical compositions and physical properties of spinach from different habitats in Khuzestan. These variations may be attributed to environmental factors such as soil composition, temperature, humidity, and geographical conditions, all of which influence spinach growth and composition. This study provides valuable insights into the impact of environmental conditions on food quality and can help optimize cultivation practices in different regions.

1.Introduction

Spinach (*Spinacia oleracea* L.) is one of the most important leafy vegetables worldwide, widely recognized for its nutritional value and medicinal properties. It belongs to the Chenopodiaceae family, which encompasses 105 genera and about 1400 species with global distribution (1). In Iran, spinach is cultivated extensively across various regions, and its wild types are abundant in foothills such as the northern slopes of Alvand, the southern Alborz range, and the Zagros Mountains. The country's climatic diversity enables detailed investigations into the phytochemical properties and nutritional composition of spinach under varying environmental conditions (2). China leads global spinach production with 18,782,961 tons—accounting for approximately 89.9% of global output. The United States, Japan, Turkey, and Indonesia follow, with the U.S. producing 4,093,060 tons, ranking second globally. Iran produces 105,351 tons of spinach (0.5% of global production), ranking seventh (3). Spinach is consumed both fresh and processed and is an excellent source of vitamins A, C, E, calcium, iron, and antioxidant compounds—playing pivotal roles in immune system enhancement, chronic disease prevention, and oxidative damage reduction. Research has shown that spinach ranks highest among vegetables for its oxygen radical absorbance capacity (ORAC).(4, 5 ,6). Spinach leaves contain approximately 3% protein and 3% mineral content—including iron, omega-3 fatty acids, potassium, and high levels of calcium (albeit in a form poorly absorbed by the human body) (7). Notably, Iranian spinach has a much lower oxalic acid content compared to foreign cultivars.(4). Fresh spinach leaves contain about 20% ascorbic acid, carotenoids including β-carotene (pro-vitamin A) and lutein, folate, phylloquinone (vitamin K), and α-tocopherol (vitamin E).(8). Its protein content (~3.2%) has been linked to cholesterol-lowering effects (9). The lipid content (~0.6%) includes important fatty acids like α-linolenic acid (omega-3) and linoleic acid (omega-6).(10). Spinach is also rich in anticancer antioxidants such as β-carotene and lutein, with β-carotene shown to improve lung function and reduce diabetes risk (9). Environmental factors—such as soil type, precipitation, light intensity, and ambient temperature—significantly affect the accumulation of primary and secondary metabolites in plants. These include chlorophylls, carotenoids, flavonoids, and phenolic compounds, which are essential to both plant physiology and human health. Moreover, the accumulation of heavy metals due to environmental conditions can influence the safety and quality of plant-based foods. Considering Khuzestan's ecological diversity, this study was designed to assess phytochemical variation, nutritional properties, and heavy metal accumulation in spinach from various locations within the province. The findings can improve understanding of environmental impacts on the quality of medicinal and edible plants and provide strategies for optimizing cultivation under similar climatic conditions.

2-Materials and Methods

Sample Collection and Preparation of Spinach

Spinach plants were collected in the winter season of 2021 from six cities within Khuzestan Province: Abadan, Shadegan, Ramhormoz, Hamidieh, Shush, and Dezful. The botanical identity of the collected *Spinacia oleracea L.* specimens was confirmed by the Department of Biology, Faculty of Science, Shahid Chamran University of Ahvaz. For phytochemical analyses, the leaves were immediately frozen and subsequently homogenized using a mortar.

Extraction by Maceration Method

Extraction was performed using a shaker. For each sample, 1.3 g of fresh spinach tissue was mixed with 15 mL of 70%

ethanol and left at room temperature for 24 h. The resulting suspension was centrifuged and filtered to obtain the extract.

Determination of Photosynthetic Pigments

Photosynthetic pigment content (chlorophylls and carotenoids) was measured using the Lichtenthaler method. Absorbance of the extracts was recorded at 470 nm, 663 nm, and 646 nm using a spectrophotometer. Concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids were calculated using the following equations:(11).

Chl a (µg/ mL) = (12.25 A663.2) - (2.79 A646)
Equation 1

Chl b (µg/mL) = (21.50 A646.8) - (5.1 A663)
Equation 2

Chl T (µg/mL) = Chl a + Chl b
Equation 3

Carotenoid (µg/mL) = (1000 A470 - 1.82Chl a - 85.02 Chl b) / 198
Equation 4

Where *W* is the fresh weight of the plant and *V* is the extract volume

Determination of Anthocyanin Content

Anthocyanin content was determined using the modified method of Cartea et al. To 2 mL of spinach extract, 10 mL of acidified methanol and 1 mL of hydrochloric acid were added. After centrifugation, absorbance was read at 550 nm. The molar extinction coefficient used was 33,000 L/mol·cm. (12).

$A = \varepsilon bc$
Equation 5

Phytochemical Compound Analysis

Total Carbohydrate was assessed using the phenol-sulfuric acid method: 1 mL of extract was mixed with 0.5 mL of 5% phenol and 2.5 mL of 98% sulfuric acid. Absorbance was measured at 490 nm after 45 min.

Total Protein was determined using the Lowry method: 0.5 mL phosphate-buffered extract (pH 7) was mixed with 2.5 mL ABC reagent. After 15 min of incubation at room temperature, 1.5 mL Folin-Ciocalteu reagent was added. Absorbance was measured at 660 nm after 45 min (14).

Reagent Formulations:

- Folin Reagent 2 N: 5 mL of Folin reagent, 50 mL of distilled water.
- Reagent A: NaOH (2 g), Na_2CO_3 (10 g), distilled water to 100 mL (This solution can be stored for 24 to a maximum of 48 h, and if precipitation or turbidity occurs, it should not be used).
- Reagent B: $CuSO_4 \cdot 5H_2O$ (1 g), H_2SO_4 (0.25 mL), distilled water to 25 mL.
- Reagent C: Potassium sodium tartrate (4.91 g), distilled water to 100 mL.
- Reagent ABC: 15 mL A + 0.75 mL B + 0.75 mL C.

Total phenolic content was determined using the method of Slinkard and Singleton with the Folin–Ciocalteu assay. A mixture was prepared by combining 0.2 mL of the extract, 1.5 mL of 5% sodium carbonate, and 1.5 mL of 10% Folin reagent. The solution was kept in the dark for 60 min, after which the absorbance was measured at 760 nm using a UV-Vis spectrophotometer(15). For total flavonoid content, the method of Chang et al. was used. 1 mL of extract was mixed with 1 mL of 2% aluminum chloride hexahydrate and 1.5 mL of 5% sodium acetate trihydrate solution. The mixture was incubated in the dark for 150 min, and absorbance was read at 440 nm using a spectrophotometer (16).

To determine total flavonol content, the method of Kumaran et al. was applied. 0.5 mL of extract was mixed with 1.5 mL of 96% ethanol and 0.1 mL of 10% aluminum chloride hexahydrate. Then, 0.1 mL of 1 M sodium acetate trihydrate and finally 2.8 mL of distilled water were added to the mixture. The solution was kept in the dark for 30 min, and absorbance was measured at 430 nm (17).

Determination of Vitamins in Spinach Samples

Vitamin C (Ascorbic Acid) was measured using the method of Hernández et al. based on titration with 0.1% 2,6-dichlorophenol-indophenol (DCIP). One milliliter of spinach extract was diluted to 5 mL with distilled water, shaken for 60 min, and then titrated after the addition of five drops of 5% orthophosphoric acid. The endpoint was the appearance of a faint pink color. Results were expressed as mg of ascorbic acid per 100 g of fresh weight (18). Vitamin E (α-Tocopherol) content was determined based on the method by Pilar Prieto. One gram of spinach tissue was mixed with 10 mL of *n*-hexane and centrifuged in the dark for 15 min. From the supernatant, 3 mL was transferred to another tube and analyzed by HPLC using Vit E Acculite CLIA Kits (Monobind Inc., USA).(19). Vitamin B_{12} was analyzed using the Komadha method. Four grams of spinach were mixed with 24 mL of acetate buffer (0.31 g sodium acetate, 0.92 g acetic acid, pH = 4.0), 1 mL sodium cyanide solution, and 0.25 g α-amylase. After incubation at 42°C for 30 min and pH adjustment to 4.8, the mixture was further incubated at 98°C for 30 min and then centrifuged at 4°C, 4000 rpm for 15 min. The supernatant was analyzed using Vit B_{12} Acculite CLIA Kits (Monobind Inc., USA) via ELISA (DANA model) (20). Total Folic Acid was extracted similarly to free folate: 5 g of spinach was homogenized in 10 mL phosphate buffer (1% ascorbic acid), then α-amylase (500 µL), protease (7.5 mL), and mouse serum (2 mL) were added. The mixture was analyzed using the Folate Acculite ELISA Kit (Monobind Inc., USA) via ELISA (21).

Determination of Heavy Metals and Mineral Elements

The concentrations of calcium (Ca), copper (Cu), iron (Fe), magnesium (Mg), zinc (Zn), cesium (Cs), lead (Pb), and cadmium (Cd) were measured using Inductively Coupled Plasma (ICP) spectrometry, following the method of Daniel et al. In this method, 0.5 g of dried aerial plant tissue was accurately weighed and finely powdered. The powdered sample was transferred to a 100 mL beaker, and 10 mL of 65% nitric acid (HNO_3) was added. To digest the plant material and initiate color change to brown, the mixture was kept under a fume hood for 24 h. After this initial digestion, the beaker was placed on a hot plate at 90°C to drive off acid vapors. Once cooled, 1 mL of 30% hydrogen peroxide (H_2O_2) was added to the mixture to further clarify the solution. The mixture was reheated to ensure complete removal of brown acidic vapors. After cooling again, the digested solution was filtered using filter paper, and the filtrate was brought to a final volume with distilled water in a 25 mL volumetric flask. The final solution was analyzed by ICP to determine the concentrations of the aforementioned heavy metals. Results were expressed as micrograms per gram (µg/g) of dry weight (22).

Antioxidant Capacity via ABTS Radical Scavenging Assay

The ABTS radical scavenging assay was conducted according to the method of Re et al. To prepare the ABTS stock solution, first 5 mL of an ABTS solution (prepared by dissolving 96.02 mg of ABTS in 25 mL of acetate buffer, pH = 4.5; buffer composition: 0.23 g sodium acetate, 0.2 mL acetic acid, and 50 mL distilled water) was mixed with 5 mL of potassium persulfate solution (prepared by dissolving 17 mg of potassium persulfate in 25 mL of acetate buffer). The mixture was incubated in the dark at 4°C for 12–16 h to generate the $ABTS•^+$ radical cation. The resulting stock solution was then diluted at a 1:16 ratio (i.e., 150 mL of ABTS stock solution with 2400 mL of acetate buffer) until its absorbance reached 0.70 ± 0.02 at 734 nm. Next, 0.2 mL of prepared extract was added to 2.8 mL of the diluted $ABTS•^+$ solution. After 6 minutes of incubation, the absorbance was measured at 734 nm using a UV-Vis spectrophotometer (23).

% Inhibition = [(Abs_control − Abs_sample) / Abs_control] × 100 Equation 6

Measurement of Electrical Conductivity

Electrical conductivity (EC), an indicator of electrolyte leakage from plant tissues, was assessed using the method of Hampton: Ten 6-mm leaf disks from spinach were placed in 15 mL Falcon tubes, each containing 15 mL of distilled water. Samples were incubated at room temperature for 24 h. EC was measured in µS/cm·g using a conductivity meter (24).

Determination of Relative Moisture Content

To determine the moisture content of spinach leaves, a whole leaf from each sample was placed in an oven at 100°C for 2 h. After the specified time, the samples were removed from the oven and weighed. The moisture percentage, based on fresh weight, was calculated using Equation 1, and the results were expressed as percentage (%) (25).

Moisture (%) = 100 × [(Fresh weight − Dry weight) / Fresh weight] Equation 7

Statistical Analysis

All experiments were conducted using a completely randomized block design with three replicates. Data were analyzed using SPSS version 20.0 (SPSS Inc., Chicago, USA). Results were expressed as mean ± standard deviation (SD). Analysis of Variance (ANOVA) was used, and statistical significance was set at $P < 0.05$.

3-Results and Discussion

Photosynthetic Pigments in Spinach from Various Habitats of Khuzestan

The evaluation of mean chlorophyll levels and associated pigments in spinach samples collected from various habitats across Khuzestan Province revealed statistically significant differences. The highest concentration of chlorophyll a was observed in spinach samples from Hamidieh and Shadegan, whereas the lowest levels were measured in samples from Abadan, Dezful, and Ramhormoz ($p < 0.05$) (Figure 1-A). Similarly, the comparison of chlorophyll b content indicated that the highest level occurred in Hamidieh, while Ramhormoz, Abadan, and Dezful exhibited the lowest concentrations. However, the differences between Shush and Shadegan were not statistically significant ($p < 0.05$) (Figure 1-B). The analysis of carotenoids showed that the lowest content was found in Hamidieh, while no significant differences were detected among the other habitats (except Hamidieh) ($p < 0.05$) (Figure 1-C). Finally, the results for anthocyanins revealed that the highest level was recorded in Ramhormoz, whereas the lowest concentrations were found in Abadan and Dezful. No significant differences in anthocyanin levels were observed among Shush, Hamidieh, and Shadegan ($p < 0.05$) (Figure 1-D). Carotenoid pigments serve two essential functions in plants: they harvest light energy that can be utilized in photosynthesis, and they protect chlorophyll from photodegradation under conditions of high light intensity. Flavonoids are secondary metabolites that contribute to plant defense mechanisms, offering protection against ultraviolet (UV) radiation, pathogens, and herbivorous organisms. These compounds exhibit potent antioxidant properties and play a key role in regulating enzymatic activities and in the biosynthesis of primary metabolites.

The concentration of flavonoids varies among plant species and is influenced by factors such as growth stage, tissue type, variety, and environmental stresses including UV radiation, drought, soil conditions, tillage, pests, diseases, and fertilizer application. In general, there is a significant positive correlation between chlorophyll a and chlorophyll b, total chlorophyll, flavonoid content, anthocyanin levels, and precipitation rate (26,27). Reports indicate that an increase in photosynthesis, chlorophyll content, Rubisco enzyme activity, biomass production, leaf growth and development, can lead to a corresponding increase in essential oil yield. In a study investigating the impact of environmental factors such as slope aspect and soil properties on the essential oil composition of *Stachys inflata* (Marjam-e-Nokhodi) in the pastures of Namarestagh, Amol, it was found that the eastern slope produced higher levels of essential oil compounds compared to the western and northern slopes. Moreover, populations from Isfahan, Mazandaran, Qaemshahr, and Yasuj were found to be richer in valuable biochemical traits of their extracts, including antioxidant activity, phenolic compounds, total flavonoids, and carbohydrates. These findings confirm that environmental factors can significantly influence photosynthetic pigment levels, including chlorophyll, thereby affecting the plant's biochemical profile and medicinal value (28,29). Saeidi et al. reported that the total carotenoid content in the medicinal plant wild rose (*Rosa canina*) varies significantly across different regions of Iran. In their study, the highest carotenoid level was recorded in samples from Kurdistan (Divandarreh), while the lowest was observed in Boyer-Ahmad (Meymand). They concluded that climatic conditions, geographical factors, and soil characteristics can substantially influence the levels of photosynthetic pigments, including carotenoids, in medicinal plant species (30).

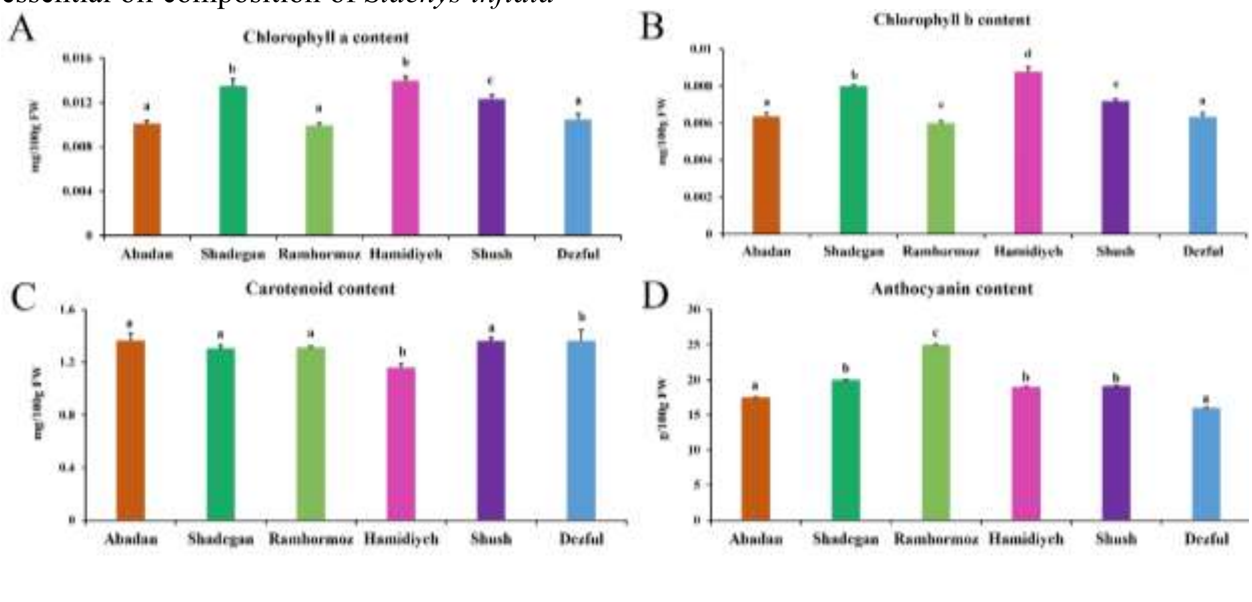

Figure 1. a) Chlorophyll a content, b) Chlorophyll b content, c) Carotenoid content, d) Anthocyanin content in spinach samples collected from various habitats in Khuzestan Province. The reported values represent the mean

± standard error based on three replicates. Identical letters above the bars indicate no significant differences between the means.

Analysis of Phytochemical Composition in Spinach from Various Habitats of Khuzestan

The results of analyzing the mean carbohydrate and total protein content in spinach samples collected from various habitats across Khuzestan Province revealed statistically significant differences. The highest carbohydrate content was observed in samples from Hamidieh, while the lowest was recorded in Ramhormoz. Following Hamidieh, spinach samples from Shush, Shadegan, Abadan, and Dezful showed progressively lower carbohydrate levels. However, there was no statistically significant difference between the mean carbohydrate contents of Dezful and Abadan, as well as between Shush and Shadegan ($p < 0.05$) (Figure 2-A). Regarding total protein content**,** the lowest level was found in spinach samples collected from Shadegan, while no significant differences were observed among the other cities **(**$p < 0.05$**)** (Figure 2-B).

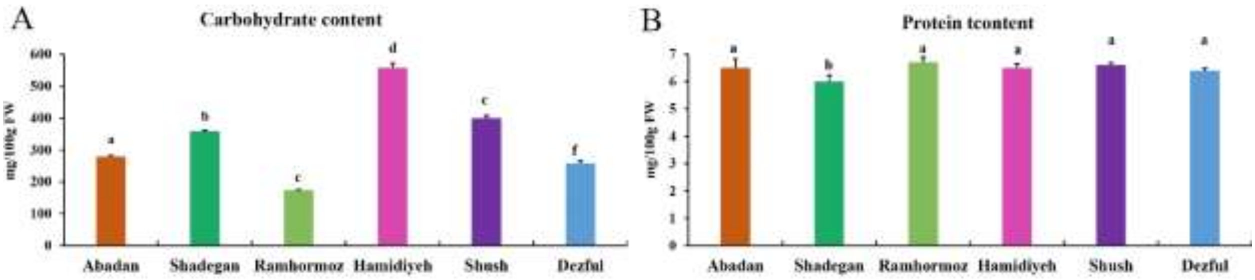

Figure 2. a) Total carbohydrate content, b) Total protein content in spinach samples collected from various habitats in Khuzestan Province. The reported values represent the mean ± standard error based on three replicates. Identical letters above the bars indicate no significant differences between the means.

The analysis of mean total phenol, flavonoid, and flavonol content in spinach samples collected from different habitats across Khuzestan Province revealed statistically significant differences**.** The highest total phenol content was observed in samples from Hamidieh**,** while the lowest values were recorded in samples from Shadegan and Dezful. No significant difference in total phenol content was detected among the samples from Abadan, Ramhormoz, and Shush ($p < 0.05$) (Figure 3-A). Regarding flavonoid content, the highest concentration was again found in Hamidieh, while the lowest was observed in Ramhormoz. After Hamidieh, the cities of Abadan, Shush, Dezful, and Shadegan showed higher flavonoid levels in descending order. However, the mean flavonoid contents in Dezful, Shadegan, and Shush were not significantly different ($p < 0.05$) (Figure 3-B). The flavonol content also varied significantly between habitats. The highest level was observed in Abadan, and the lowest in Shadegan. Samples from Dezful, Shush, Hamidieh, and Ramhormoz contained progressively lower amounts. Nevertheless, no significant difference was found between Shush and Hamidieh in terms of flavonol content ($p < 0.05$) (Figure 3-C).

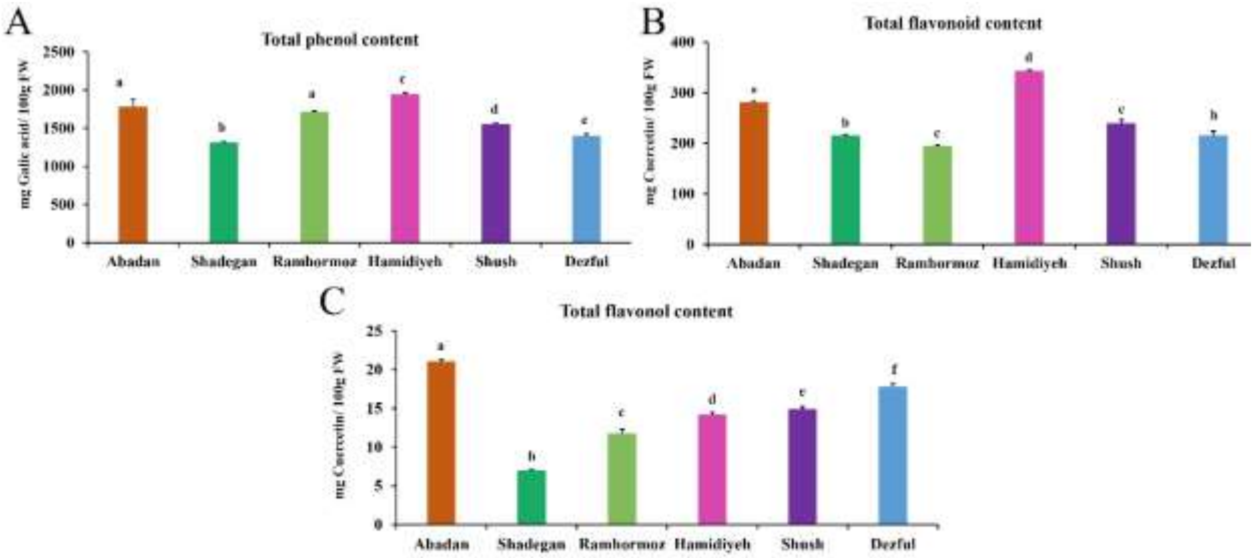

Figure 3. a) Total phenol content, b) Total flavonoid content, c) Total flavonol content. The reported values represent the mean ± standard error based on three replicates. Identical letters above the bars indicate no significant differences between the means.

The analysis of the chemical composition of Ferula assa-foetida essential oil in natural habitats of Kerman Province revealed that the production of bioactive plant compounds may result from the activation of secondary metabolite biosynthetic pathways, which manifest differently across various plant species. In contrast, primary metabolites such as carbohydrates, proteins, and lipids generally occur in similar forms across all plants. These findings suggest that the biosynthesis of secondary metabolites is highly influenced by the plant's environmental conditions (31). The highest accumulation of hypericin in Hypericum perforatum was observed when the plant developed in areas with high relative humidity. This suggests that the chemical composition of medicinal plants is not constant across different rangelands and is significantly influenced by environmental conditions. In general, the production of bioactive compounds in plants is closely linked to developmental processes, including metabolic changes, morphological adaptations, and tissue differentiation. Findings from this study indicate that at higher altitudes, due to lower temperatures—which act as environmental stressors disrupting physiological balance—there is an increase in phenolic compounds, secondary structures such as lignin and suberin, and anthocyanins in plants (32). Bezanjani et al. (2017) investigated the effect of altitude on total phenol and flavonoid content in Crataegus microphylla, and reported that this species exhibited the highest levels of these compounds at 1000 meters elevation, compared to plants growing at lower altitudes (31). A comparative study on the phytochemical composition of Epilobium minutiflorum across different elevations further demonstrated that its high antioxidant capacity was attributable to elevated concentrations of flavonoids and phenolic compounds (33). According to the results of regression analysis across different species, a significant relationship was observed between total flavonoid content, antioxidant capacity, total phenolic compounds, and altitude above sea level. However, no correlation was found between anthocyanin levels and elevation. Researchers emphasized that environmental factors, particularly high altitude, can lead to an increase in flavonol accumulation within plant tissues (34). The regression analysis revealed a significant correlation between 30 identified compounds in populations of *Epilobium minutiflorum* and altitude above sea level. Among these, most of the positively correlated compounds belonged to the classes of flavonoids, phenolics, steroids, and terpenes. In other words, the increased concentration of these 30 compounds—particularly flavonoids, phenolic acids, and steroids—appeared to be associated with elevation gain. Furthermore, the results obtained from GC/MS analysis showed strong agreement with those from spectrophotometric assays, confirming the observed phytochemical trends (35). Jahanshahi et al. investigated the effects of aerial part stress on four wild almond (Amygdalus spp.) species. Their study revealed that increasing elevation leads to a decrease in vegetative growth parameters and biomass accumulation (36). Moreover, seedlings at lower elevations exhibited greater growth, while those from higher altitudes showed significantly reduced development. Additionally, an increase in sodium chloride (NaCl) concentration and altitude caused a decline in plant pigment content (37). In a related study, Zakarianejad et al**.** examined the effects of altitude and soil characteristics on secondary metabolites in various organs of *Viola odorata* (sweet violet) across natural habitats in Mazandaran Province. They concluded that the most influential ecological factors affecting the quantity and quality of bioactive compounds in medicinal plants include: Climatic conditions (temperature, light intensity, precipitation, wind), Soil properties (nutrient content, texture, pH), Geographic factors (altitude, slope, and aspect) (38). Furthermore, a phytochemical analysis of the multipurpose medicinal plant *Withania coagulans* in natural habitats of Sistan and Baluchestan Province showed that quantitative differences in phytochemical compounds—such as phenolics, flavonoids, and anthocyanins—across populations may be attributed to genetic variation or ecological conditions specific to each habitat. Additionally, it appears that differences in secondary metabolite content among habitats with similar geographic and ecological characteristics are likely due to intraspecific genetic diversity (39).

Assessment of Vitamin Content in Spinach from Various Habitats of Khuzestan

The analysis of mean concentrations of vitamins C, E, B_{12}, and folic acid in spinach (*Spinacia oleracea*) samples collected from various habitats across Khuzestan Province revealed statistically significant differences. The highest vitamin C content was observed in samples from Abadan, while the lowest was recorded in Ramhormoz. No significant differences were found between Dezful and Shadegan ($p < 0.05$) (Figure 4-A). The results also showed that the highest levels of vitamin E were found in spinach samples from Dezful and Hamidieh, whereas lower levels were detected in samples from Abadan, Shadegan, Ramhormoz, and Shush ($p < 0.05$) (Figure 4-B). The highest vitamin B_{12} content was found in samples from Ramhormoz and Shush, and the lowest in Abadan. Among the remaining locations, no significant difference was observed in the mean vitamin B_{12} levels between Dezful, Hamidieh, and Shadegan ($p < 0.05$) (Figure 4-C). As for folic acid, the highest concentration was recorded in samples from Ramhormoz, and the lowest in Abadan and Dezful. However, there were no significant differences in folic acid content among Shush, Hamidieh, and Shadegan ($p < 0.05$) (Figure 4-D).

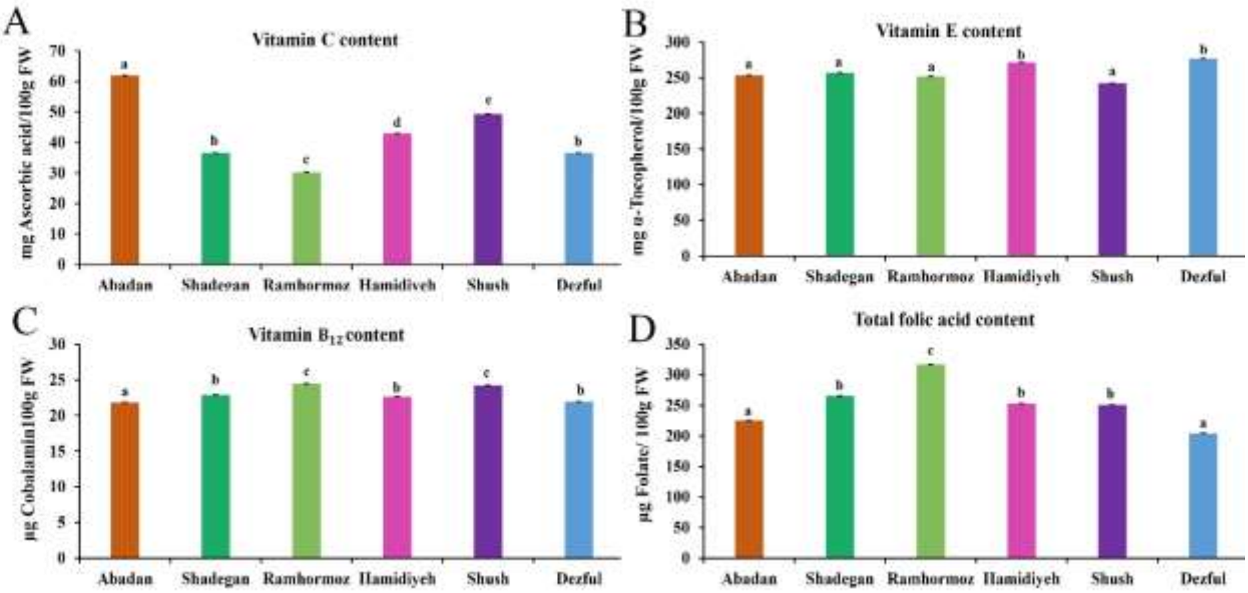

Figure 4. a) Vitamin C content, b) Vitamin E content, c) Vitamin B12 content, d) Folic acid. The reported values represent the mean ± standard error based on three replicates. Identical letters above the bars indicate no significant differences between the means.

Assessment of Heavy Metals and Mineral Elements in Spinach from Various Habitats of Khuzestan

The analysis of mean concentrations of mineral elements—namely calcium (Ca), copper (Cu), iron (Fe), and magnesium (Mg)—in spinach (*Spinacia oleracea*) samples collected from various habitats of Khuzestan Province revealed statistically significant differences. The highest calcium content was recorded in samples from Dezful, while the lowest levels were observed in samples from Hamidieh and Shadegan. However, no significant differences were detected between Shush and Abadan, as well as between Hamidieh and Shadegan ($p < 0.05$) (Figure 5-A). In the case of copper, the highest content was found in samples from Shush, while the lowest was observed in Ramhormoz. The mean copper concentrations in Abadan, Shadegan, and Hamidieh did not differ significantly ($p < 0.05$) (Figure 5-B). The iron content was highest in Dezful and lowest in Hamidieh, although no significant differences were observed between Shush and Abadan, or between Shadegan and Ramhormoz ($p < 0.05$) (Figure 5-C). Regarding magnesium, the highest concentration was found in samples from Ramhormoz, while the lowest was measured in Hamidieh and Shadegan. There was no significant difference in magnesium content between Shush and Abadan, as well as between Hamidieh and Shadegan ($p < 0.05$) (Figure 5-D).

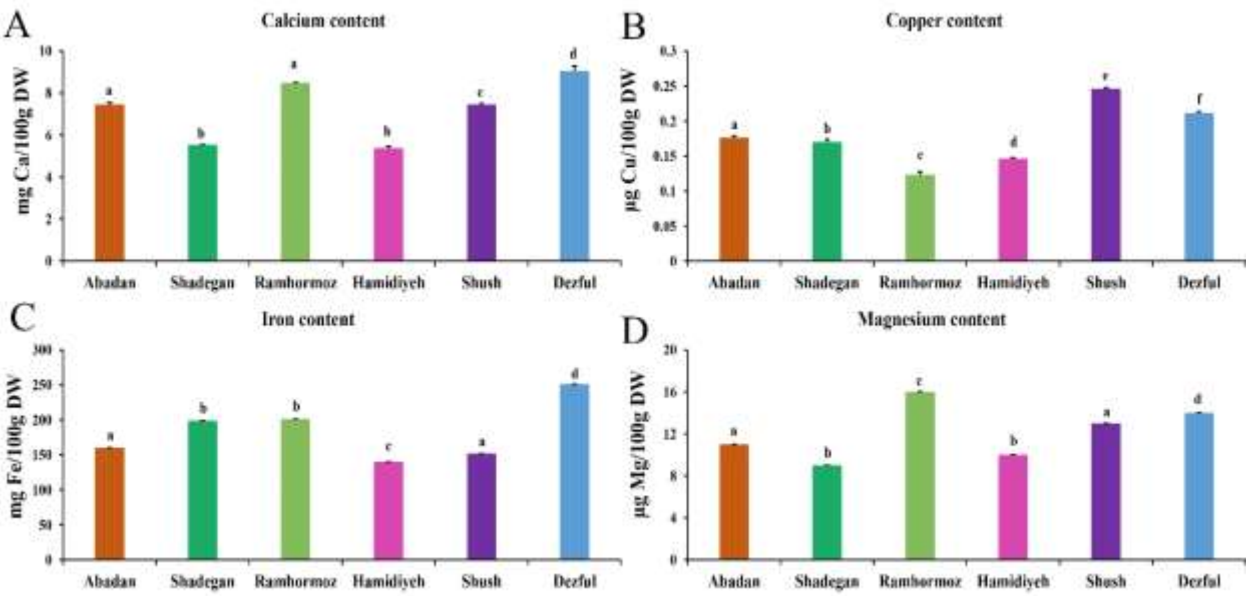

Figure 5 .a) Calcium content, b) Copper content, c) Iron content, d) Magnesium content. The reported values represent the mean ± standard error based on three replicates. Identical letters above the bars indicate no significant differences between the means.

The analysis of mean concentrations of heavy metals, including zinc (Zn), cesium (Cs), and lead (Pb), in spinach samples collected from different habitats in Khuzestan Province revealed statistically significant differences. The highest zinc content was recorded in samples from Abadan, while the lowest levels were observed in Shadegan and Hamidieh. However, no significant difference was found in zinc content between Shadegan and Hamidieh, as well as between Dezful and Ramhormoz ($p < 0.05$) (Figure 6-A). The highest cesium concentration was observed in spinach samples from Abadan, and the lowest in Hamidieh. Meanwhile, no significant difference in cesium content was detected between samples from Dezful and Ramhormoz ($p < 0.05$) (Figure 6-B). As for lead (Pb), the highest concentration was measured in spinach from Abadan, and the lowest in Hamidieh. However, no significant differences were observed in mean lead content between samples from Dezful and Ramhormoz, as well as between Shush and Shadegan ($p < 0.05$) (Figure 6-C).

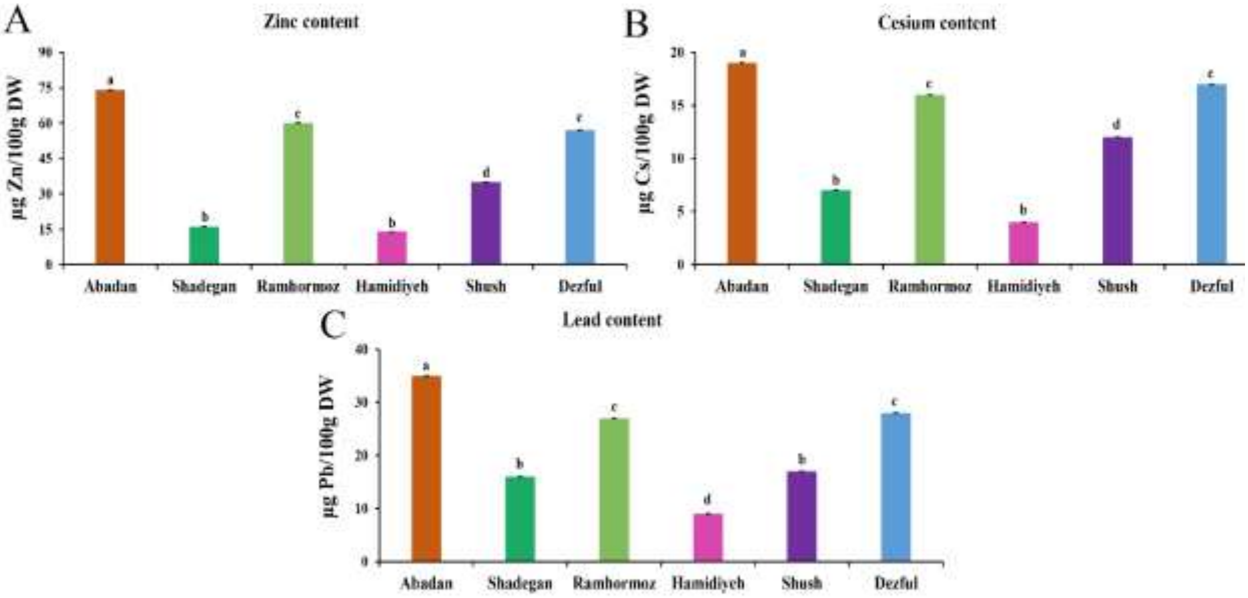

Figure 6. a) Zinc content, b) Cesium content, c) Lead content. The reported values represent the mean ± standard error based on three replicates. Identical letters above the bars indicate no significant differences between the means.

Dejampour et al. (2012) examined the effects of salt stress on almond (Prunus spp.), reporting that increased soil salinity led to a decrease in the uptake of essential elements such as copper, zinc, iron, manganese, and potassium, and an increase in the uptake of magnesium, sodium, chloride, nitrogen, phosphorus, and calcium. The elevated absorption of sodium and chloride ions impairs the uptake of essential nutrients and induces ionic toxicity in the plant. Thus, the reduction in copper, zinc, iron, manganese, and potassium is likely due to salinity-induced competition with sodium and chloride. Such increased uptake of Mg, Cl, Na, and Ca, along with decreased K uptake, in response to salt stress, has previously been documented (40). Additionally, it has been suggested that calcium plays a regulatory role by buffering membrane leakage caused by salinity, implying that habitats with higher heavy metal content may also be associated with saline soils (41). In seedling growth studies, it was shown that seedlings originating from lower altitudes exhibited greater growth and stress tolerance, whereas those from higher altitudes accumulated more toxic elements such as sodium and chloride, indicating greater sensitivity to salinity (42). Investigations into morphological diversity, phenolic content, and antioxidant activity of different populations of Nepeta species from natural habitats in Ardabil and Azerbaijan provinces demonstrated that, among living organisms, plants are particularly vulnerable to UV radiation due to their dependence on sunlight for photosynthesis. It is therefore suggested that the increase in phenolic compounds at higher altitudes is a defensive response to elevated UV exposure. It has also been established that light intensity, photoperiod, and temperature significantly affect the biosynthesis of many secondary metabolites. Moreover, antioxidant performance varies with several factors, including plant maturity, climatic conditions, plant part used, harvest timing, post-harvest storage, and preservation methods. Importantly, aside from environmental factors, genetic variation plays a key role in determining the quantitative and qualitative traits of medicinal and aromatic plants (43,44).

Evaluation of ABTS Radical Scavenging Capacity in Spinach from Various Habitats of Khuzestan

The comparison of mean antioxidant capacity, assessed through the ABTS radical scavenging assay, in spinach samples collected from various habitats in Khuzestan Province revealed statistically significant differences. Specifically, the lowest antioxidant capacity was recorded in samples from Shadegan. It is also noteworthy that no significant differences in antioxidant capacity were observed among the other studied habitats ($p < 0.05$) (Figure 7).

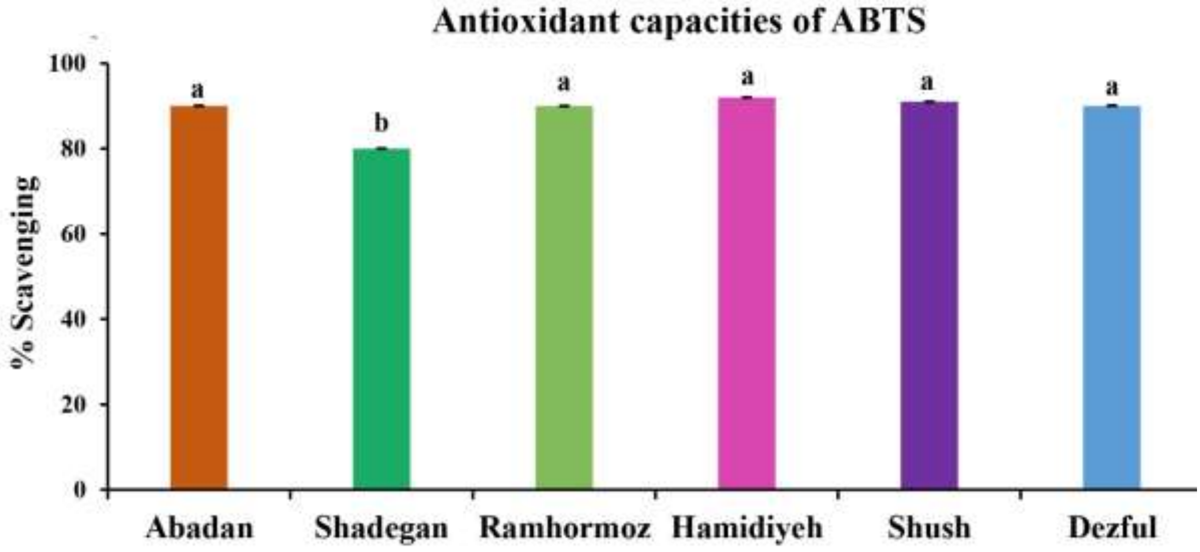

Figure 7. ABTS inhibition activity. The reported values represent the mean ± standard error based on three replicates. Identical letters above the bars indicate no significant differences between the means.

Evaluation of Electrical Conductivity in Spinach from Various Habitats of Khuzestan

The comparison of mean electrical conductivity (EC) in spinach samples collected from various habitats of Khuzestan Province showed statistically significant differences. The highest EC value was observed in samples from Ramhormoz, while the lowest EC was recorded in samples from Dezful and Abadan. There were no significant differences in EC values among spinach samples collected from Shadegan, Shush, and Hamidieh ($p < 0.05$) (Figure 8-A).

Assessment of Relative Moisture Content in Spinach from Various Habitats of Khuzestan

The comparison of mean total relative moisture content in spinach samples collected from the studied habitats showed no statistically significant differences ($p < 0.05$) (Figure 8-B).

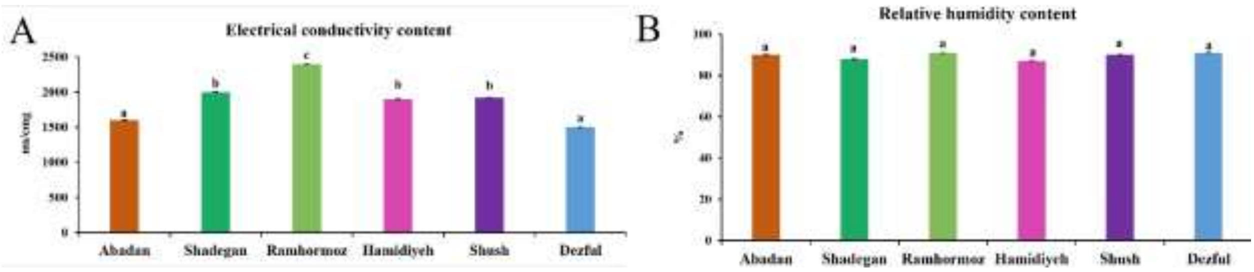

Figure 8.a) Electrical conductivity, b) Relative moisture content. The reported values represent the mean ± standard error based on three replicates. Identical letters above the bars indicate no significant differences between the means.

Electrical conductivity (EC) is a key indicator for assessing the accumulation of soluble ions in plant tissues and is widely used to evaluate the physiological and chemical status of plants. The EC of spinach is influenced by several factors, including environmental conditions, soil type, irrigation water quality, and the uptake of mineral elements.

In the present study, significant differences were observed in the EC values of spinach samples collected from different habitats of Khuzestan Province. The highest EC was recorded in samples from Ramhormoz, while the lowest was observed in Dezful.

Previous studies have shown that plants growing in arid regions or saline soils typically exhibit higher EC compared to those in low-salinity soils. This variation is likely due to chemical and physical properties of the soil. Saline soils generally contain elevated levels of soluble ions, such as sodium (Na^+) and chloride (Cl^-), which contribute to increased EC in plant tissues (45). Another important factor influencing EC is irrigation water quality. The use of water with high concentrations of dissolved salts can lead to greater ion accumulation in plant tissues. Additionally, light intensity and ambient temperature may affect EC indirectly by altering mineral uptake and transpiration rates. Studies indicate that higher transpiration in hot or high-light environments can result in increased ion concentration within plant cells (46).

Relative moisture content (RMC) in plants is a key physiological indicator that is directly influenced by environmental conditions and the internal water status of the plant. RMC reflects the amount of water present in plant tissues and plays a critical role in growth, photosynthesis, and the stability of cellular structures. In this study, no significant differences were found in the relative moisture content of spinach samples collected from different habitats across Khuzestan Province. This lack of variability may be attributed to relatively similar environmental conditions among the studied sites. Climatic factors such as temperature, air humidity, and light intensity significantly influence the plant's RMC. As a leafy vegetable, spinach typically requires a high level of soil and atmospheric humidity to maintain its succulent tissue structure. A deficiency in moisture can lead to loss of turgor pressure, resulting in leaf wilting (47). Conversely, higher relative moisture levels in plant tissues are associated with improved photosynthetic efficiency and enhanced nutrient transport (48). Effective water resource management and regular irrigation practices are critical to maintaining adequate moisture levels in plants. Although the studied regions in Khuzestan may benefit from different water sources, variation in water availability and quality could impact plant moisture content. Additionally, soil type plays a major role in water retention and supply to plants; for instance, sandy soils have lower water-holding capacity compared to clay soils, which can ultimately affect the relative moisture content over time (49).

4-General Conclusion

The results of this study demonstrated that geographical and environmental factors have a significant impact on the chemical composition and physicochemical characteristics of spinach (Spinacia oleracea) collected from various regions of Khuzestan Province. Overall, spinach samples from Hamidieh and Shadegan exhibited superior quality in terms of photosynthetic pigments (such as chlorophylls and anthocyanins), carbohydrates, proteins, and certain vitamins including vitamin C and vitamin E. These traits may reflect more optimal growing conditions in these regions. In particular, the elevated levels of chlorophyll and other beneficial compounds suggest the potential for producing high-quality, nutrient-rich crops in these habitats. In addition, findings related to heavy metals and antioxidant capacity emphasize the importance of selecting suitable habitats for spinach cultivation, as these environmental variables can strongly influence the health-promoting properties of the crop. Based on the overall findings, it can be concluded that spinach from Hamidieh and Shadegan displayed the highest overall quality, particularly with regard to photosynthetic pigments, vitamins, carbohydrates, and beneficial proteins. These areas also showed more favorable levels of mineral elements and lower concentrations of heavy metals.

Among other regions, Dezful and Abadan also showed promising results, particularly in terms of calcium, iron, and selected vitamins. Conversely, Shadegan ranked lowest in terms of antioxidant capacity and some other biochemical traits, possibly reflecting adverse environmental effects on the spinach grown in that area. Therefore, based on the outcomes of this research, Hamidieh and Shadegan can be considered suitable regions for producing high-quality spinach with enhanced nutritional content, making them ideal choices for health-conscious cultivation. These insights can serve as a practical guide for selecting optimal cultivation zones to maximize the health and nutritional value of spinach for consumers.

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مقاله علمی_پژوهشی

ارزیابی ارزش غذایی و ویژگی‌های فیتوشیمیایی گیاه اسفناج (*Spinacia oleraceae*L.) در رویشگاه‌های مختلف استان خوزستان

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چکیده

این مطالعه به بررسی میزان رنگدانه‌های فتوسنتزیو ترکیبات شیمیایی نمونه‌های اسفناج جمع‌آوری‌شده از رویشگاه‌های مختلف استان خوزستان پرداخته است. نتایج نشان داد که مقادیر کلروفیل‌ها و سایر رنگدانه‌ها در این نمونه‌ها تفاوت‌های معناداری داشته است. بیشترین میزان کلروفیل a در حمیدیه و شادگان مشاهده شد، درحالی‌که بیشترین میزان کلروفیل b در حمیدیه دیده شد. در بررسی ترکیبات شیمیایی، بیشترین میزان کربوهیدرات در حمیدیه و کمترین در رامهرمز بود. میزان پروتئین کل در شادگان کمترین مقدار را داشت. همچنین، مقادیر ویتامین‌ها و عناصر معدنی مختلف نیز در نمونه‌ها تفاوت‌های معناداری را نشان داد. بیشترین ویتامین C در آبادان، ویتامین E در دزفول و حمیدیه، ویتامین B_{12} در رامهرمز و شوش و اسیدفولیک در رامهرمز مشاهده گردید. در بررسی عناصر معدنی، بیشترین میزان کلسیم در دزفول، مس در شوش، آهن در دزفول و منیزیم در رامهرمز بود. علاوه بر این، مقادیر فلزات سنگین شامل روی، سزیم و سرب در نمونه‌های مختلف تفاوت معناداری داشتند. همچنین، ظرفیت آنتی‌اکسیدانی اسفناج جمع‌آوری‌شده از شادگان کمترین مقدار را داشت و هدایت الکتریکی اسفناج رامهرمز بیشترین مقدار را نشان داد. نتایج این مطالعه نشان‌دهنده تفاوت‌های معنادار در ترکیبات شیمیایی و ویژگی‌های فیزیکی اسفناج جمع‌آوری‌شده از رویشگاه‌های مختلف استان خوزستان است. این تفاوت‌ها می‌تواند ناشی از عوامل محیطی مانند خاک، دما، رطوبت و شرایط جغرافیایی مختلف باشد که بر رشد و ترکیبات اسفناج تأثیر می‌گذارند. این اطلاعات می‌تواند به بهبود درک ما از تأثیر محیط بر کیفیت مواد غذایی و بهینه‌سازی شرایط کشت در مناطق مختلف کمک کند.