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Proximate Composition and Nutritive Value of Some Leafy Vegetables from Faisalabad, Pakistan

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Abstract: The supply of nutrients and proximate matter is insufficient to fulfil the dietary needs of the quickly expanding human population. Green leafy vegetables can prove economical sources of minerals, vitamins and fibers to overcome nutritional deficiencies. Five leafy vegetables (*Brassica juncea*, *Spinacia oleracea*, *Trigonella foenum-graecum*, *Chenopodium album* and *Lactuca sativa*) were collected from a horticulture garden and vegetable market for the evaluation of their proximate matter and nutritional composition. Their contents of proximate (moisture, ash, fats, fibers, carbohydrates and protein), minerals (Ca^{2+}, K^+ and P^+) and anti-nutrients were examined using standard protocols. The contents of fats (0.43 mg g^{-1}f.w.) in *S. oleracea*; fibers (0.8 mg g^{-1}f.w.) in *S. olerace*; carbohydrates (0.89 mg g^{-1}f.w.); in *B. juncea*, proteins (0.91 mg g^{-1}f.w.) in *L. sativa*; and vitamin A (1.18 mg g^{-1}f.w.) in *C. album* from the horticulture garden were high enough to meet the daily dietary requirements of adults. Tannins were higher in species collected from the market, which is toxic for human health. It is concluded that vegetables of horticulture garden grown in suitable environmental conditions possess better nutritional composition as compared to vegetables purchased from markets. However, a large-scale research is needed for the analysis of vitamins, minerals, antioxidants, anti-nutrients and heavy metals in leafy vegetables. Further research on ethnomedicinal attributes of leafy vegetables is recommended.

Keywords: proximate matter; moisture; carbohydrates; proteins; tannin; anti-nutrients; antioxidants

1. Introduction

The food requirements of a rapidly growing population is increasing with the passage of time while available land resources are diminishing day by day due to aridity [1,2]. Raw as well as cooked leafy vegetables are used as the cheapest source of energy to overcome nutritional deficiencies. Furthermore, leafy vegetables are considered as part of well-balanced diets and are a source of essential nutrients such as carbohydrates, carotene, protein, vitamins, calcium, iron, ascorbic acid and minerals [3–5]. Strong antioxidants reported in green vegetables protect us from free radical which ultimately reduce the health risks such as cardiovascular diseases and cancer [6,7].

Macronutrients, i.e., carbohydrates, lipids, fats and proteins are a major portion of the human diet, which is essential for nourishment [8,9]. Almost all the plant parts such as leaves, stem, fruit, seeds and roots formed of these macronutrients, especially proteins [10]. The selection of plants for nutritive value, systematic classification and nutrient improvement programs depend upon quality and quantity of protein in seeds [11]. Some biochemicals, i.e., moisture, ash and fibers, are also reported as important for human health [12]. Nutrient-rich leafy vegetables are abundantly cultivated in different regions of world, especially in South Asia to fulfill dietary and medicinal requirement [8].

Micronutrients such as vitamins and minerals are essential dietary compounds, obtained from different edible parts of plants [13]. Vitamins can provide substantial health benefits such as strong bones and healthy teeth [14,15]. Minerals constitute 5% of total body weight, which is essential for all physiological processes and components of teeth, bones, blood and tissues [14]. Na^+ is an essential electrolyte that maintains water balance and help in balancing blood pressure level [16]. Ca^{2+} is the most abundant mineral in the human body and is available in nuts, cereals and dandelion leaves [17]. K^+ is also a crucial mineral for the human body which helps to regulate fluid balance for optimum growth and its deficiency can cause hypertension [18]. P^+ is an essential mineral which is a component of our bones and teeth, and its deficiency can cause rickets, osteomalacia and osteoporosis [19]. However, tannins are an important class of anti-nutrients, which form insoluble complexes with essential elements of the body, resulting in the reduction of food digestion capability [20].

Malnutrition is one of the most common dilemmas of public health in developing countries such as Pakistan [1]. This malnutrition leads to the possibility of chronic diseases such as stunted growth and some other nutritional disorder. Deficiency of calcium and zinc is known as global health challenge on the basis of the shortage in the food supply [21]. The number of under-nourished and stunting people is increasing with the passage of time in Pakistan [22]. Nutrient-rich vegetables are strongly associated with gastrointestinal health, vision and overall body health. Contrastingly, the deficiency of vegetables cause severe health risk by increasing the risk of ischemic heart disease by 31% and of brain stroke by 11% in men [23].

These leafy vegetables are valuable for the maintenance of good health and inhibition of several ailments. The main objective of this study was to evaluate nutritional composition of five leafy vegetables (*Brassica juncea*, *Spinacia oleracea*, *Trigonella foenum-graecum*, *Chenopodium album* and *Lactuca sativa*) collected from a horticulture garden and a vegetable market. The second objective was to investigate the nutritional potential of leafy vegetables for the fulfilment of dietary needs. There are limited studies which report the nutritional comparison of leafy vegetables gathered from two locations. Furthermore, no research was conducted previously on the proximate composition and nutritive value of leafy vegetables from the Faisalabad region. It is hypothesized that vegetables collected from more suitable locations possess better nutritional composition.

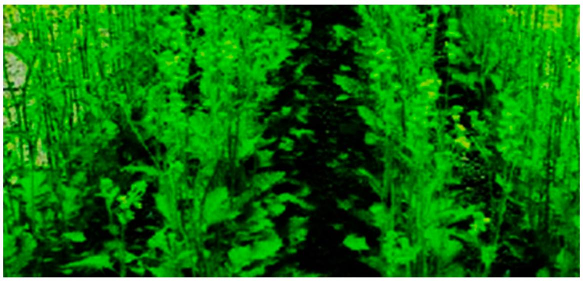
2. Material and Methods

2.1. Collection Sites

Five different species of leafy vegetables (*Brassica juncea*, *Spinacia oleracea*, *Trigonella foenum-graecum*, *Chenopodium album* and *Lactuca sativa*) were collected from two different sites of district Faisalabad, Punjab Pakistan (Figure 1). These sites include the horticulture garden of the University of Agriculture Faisalabad (Coordinates: 31.42° N, 73.09° E, Altitude: 186 m a.s.l.) and a vegetable market situated in the Jhang bazar of Faisalabad (Coordinates: 31.57° N, 73.18° E, Altitude: 190 m a.s.l.) as elaborated in Figure 2. Details of each leafy vegetable regarding local name, family name and parts used are explained in Table 1.

Table 1. Description of leafy vegetables collected for nutrients analysis.

Species Name	Local Name	Family Name	Parts Used
Brassica juncea	Saag	Brassicaceae	Leaves
Spinacia oleracea	Palak	Amaranthaceae	Leaves
Trigonella foenum-graecum	Methi	Fabaceae	Flashy leaves
Chenopodium album	Bathu	Amaranthaceae	Leaves
Lactuca sativa	Salad	Asteraceae	Shoots



Brassica juncea

Spinacia oleracea

Trigonella foenum-graecum

Chenopodium album

Lactuca sativa

Figure 1. Pictorial description of leafy vegetables, *Brassica juncea*, *Spinacia oleracea*, *Trigonella foenum-graecum*, *Chenopodium album* and *Lactuca sativa*.

Figure 2. Map of the Faisalabad District showing the horticulture garden and market (Red).

2.2. Plant Physiological and Nutritional Analysis

Each species was collected from two selected locations for physiological and nutritional analysis. Local varieties of all the leafy vegetables were selected for current investigation. Already grown leafy vegetables of the horticulture garden were harvested after two months of the sowing date, and the same leafy vegetables were purchased from market at similar vegetative stage. Three samples of each species were collected, and each sample possessed 5 to 15 leaves. In each leafy vegetable, leaf lamina separated from petiole was analyzed for nutritional composition. After that, fresh samples were rinsed with deionized water and dried at room temperature. Some samples were packed in zipper bags and preserved in a refrigerator (−80 °C) for further analysis. Some samples were oven dried at 37 °C for mineral analysis. All the physiological and nutritional analyses were conducted in the plant physiology laboratory, University of Agriculture, Faisalabad.

2.3. Determination of Photosynthetic Pigments

Leaves were collected for the determination of chlorophyll and carotenoid contents. For chlorophylls, 0.1 g of fresh leaf sample was extracted with 8 mL of 95% acetone for 24 h at 4 °C in the dark. The absorbance was measured by a spectrophotometer (UV-2550; Shimadzu, Kyoto, Japan) at 646.6, 663.6 and 450 nm. Chlorophyll content was calculated by the standard method of Arnon [24].

2.4. Determination of Proximate Contents

The proximate analyses (moisture, ash, fibers, crude fats, proteins and carbohydrates) of all the samples were determined. The moisture and ash were determined using the weight difference method. The nitrogen value, which is the precursor for protein of a substance, was determined by micro Kjeldahl method described by Pearson [25], involving digestions, distillation and finally, titration of the sample. The nitrogen value was converted to protein by multiplying a factor of 6.25. Carbohydrate content was determined by difference method. All the proximate values are reported in percentage [26,27].

2.5. Determination of Nutrients

The nutrients contents, namely, Na^+, K^+, Ca^{2+} and P^+, of the selected vegetable species was done using a mineral atomic absorption spectrometer (Perkin Elmer AA Analyst 700). The results were obtained while using a working standard of 1000 ppm for each of the species [28].

2.6. Determination of Oxalate and Tannin Content

Oxalate contents were evaluated following the titration method according to the protocol in [29] while the method of [30] was followed to examine the quantity of tannins.

2.7. Statistical Analysis

Statistical analysis of data was performed with analysis of variance (ANOVA) by using a statistical program Co-Stat version 6.2, Cohorts Software, 2003, Monterey, CA, USA. All the data obtained were tested by one-way analysis of variance (ANOVA). Thus, the differences between treatments were determined by using ANOVA, and the least significant difference test ($p < 0.05$) was used for multiple comparisons between treatment means. Logarithmic or inverse transformations were performed for data normalization, where necessary, prior to analysis. This data was also subjected to multivariate analysis (PCA) and correlation by using R software (R Core Team, 2019) to determine association between physiological and nutritional parameters.

3. Results

3.1. Photosynthetic Pigments

The leafy vegetables collected from the two different locations exhibited varied responses regarding their photosynthetic pigments (Figure 3). *Chenopodium album* from the horticulture garden surpassed all other vegetables regarding chlorophyll a, chlorophyll b, total chlorophyll and carotenoids. A constant decrease in photosynthetic pigments was observed in *Lactuca sativa* as compared to other species, except carotenoids, which showed minimum values in *Spinacia oleracea.* Chlorophyll a content exhibited the highest values in vegetables of the horticulture garden and the lowest values in the vegetables from the market. Chlorophyll b and the total chlorophyll showed similar behavior among all the leafy vegetables. The highest carotenoid content was observed in *Chenopodium album* from the horticulture garden and the lowest values were noted in *Spinacia oleracea* from the market.

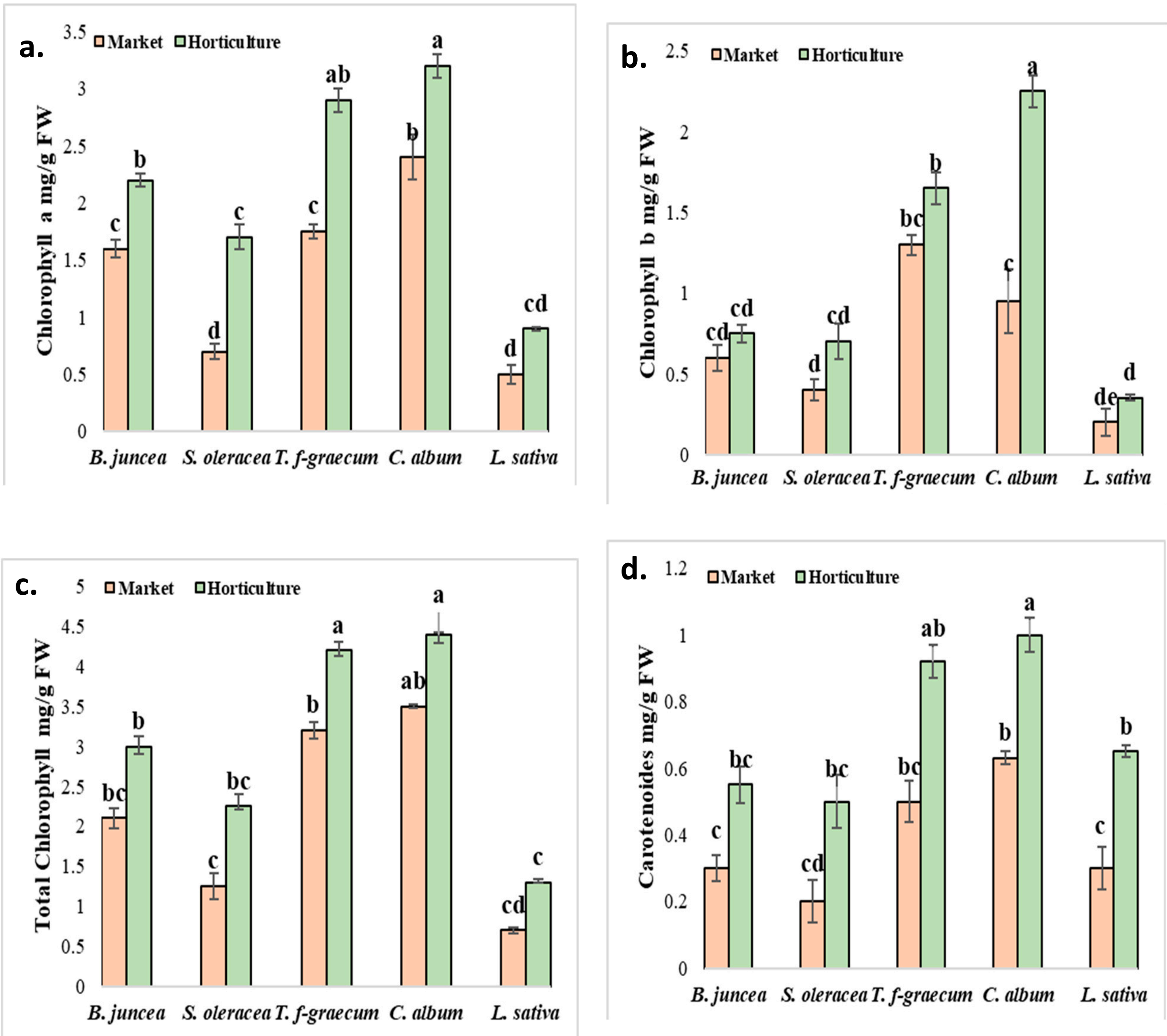


Figure 3. Chlorophyll a (**a**), Chlorophyll b (**b**), Total chlorophyll (**c**) and Carotenoids (**d**) in leafy vegetables (*Brassica juncea*, *Spinacia oleracea*, *Trigonella foenum-graecum*, *Chenopodium album* and *Lactuca sativa*) collected from two different sites. Mean ± SD, $n = 3$. One-way ANOVA was performed, and the mean difference was tested by ($p < 0.05$). Letters on error bars illustrate significant difference between parameters of different vegetables.

3.2. Proximate Contents

The proximate contents of leafy vegetables, such as moisture, ash, fats, fibers, carbohydrates and proteins are presented in Figure 4. The results revealed that vegetables of the horticulture garden dominated over the market specimens regarding all the proximate attributes. The moisture contents were in the range of 8.02 to 14.77 mg g^{-1}f.w., in which the lowest values were obtained from *Chenopodium album* from the market and the highest from *Brassica juncea* from the horticulture garden. The ash contents exhibited a similar trend as observed in the moisture contents. The lowest values for the fat and fiber contents were recorded in *Chenopodium album* from the market, while the highest values were seen in *Spinacia oleracea* from the horticulture garden. Carbohydrates varied from 0.47 to 0.89 mg g^{-1}f.w., with the maximum quantity analyzed in *Brassica juncea* from the horticulture garden and the minimum in *Trigonella foenum-graecum* from the market. The cardinal limits for the protein contents were noticed in the horticulture garden and the market samples of *Lactuca sativa*.

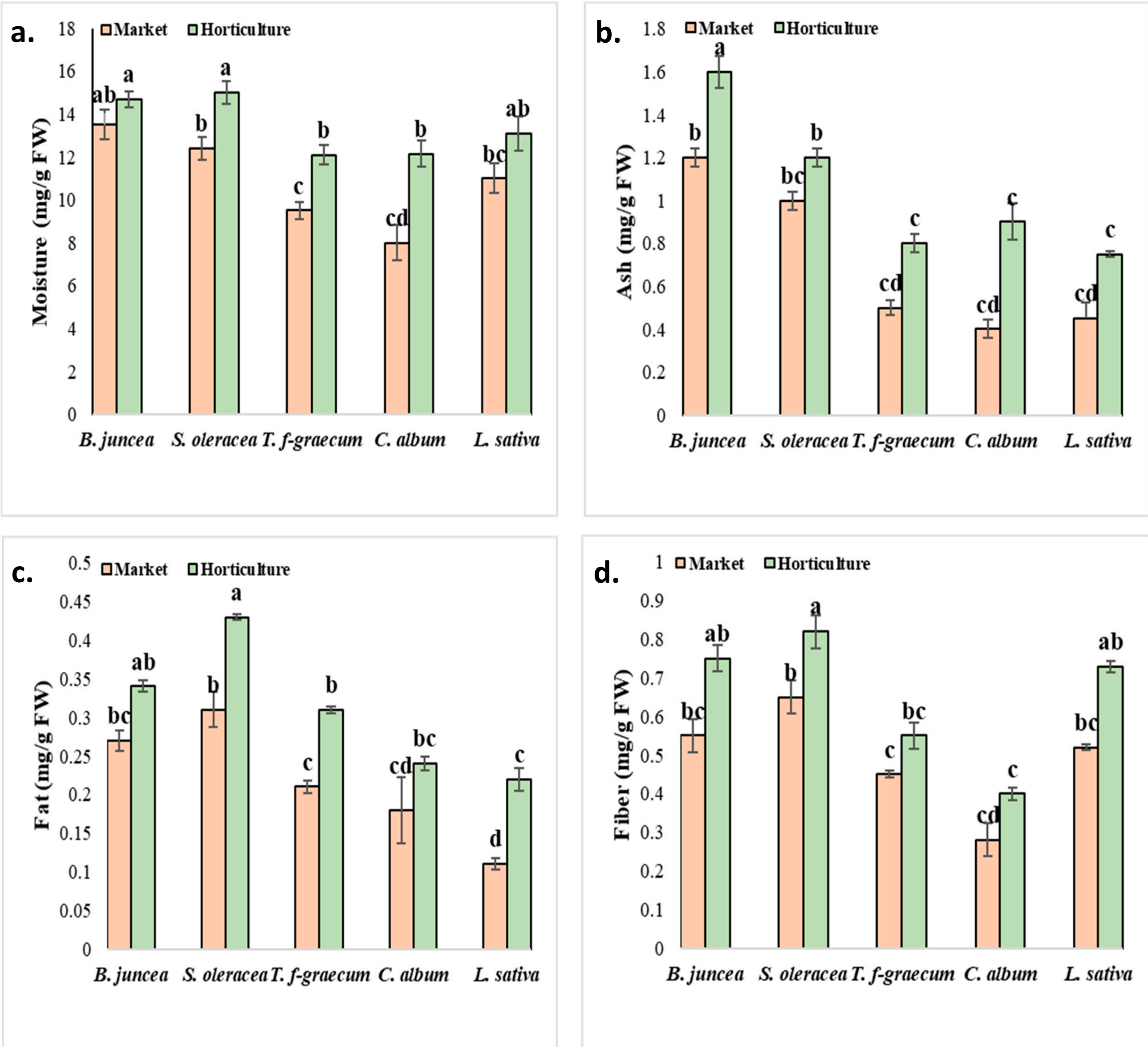


Figure 4. *Cont.*

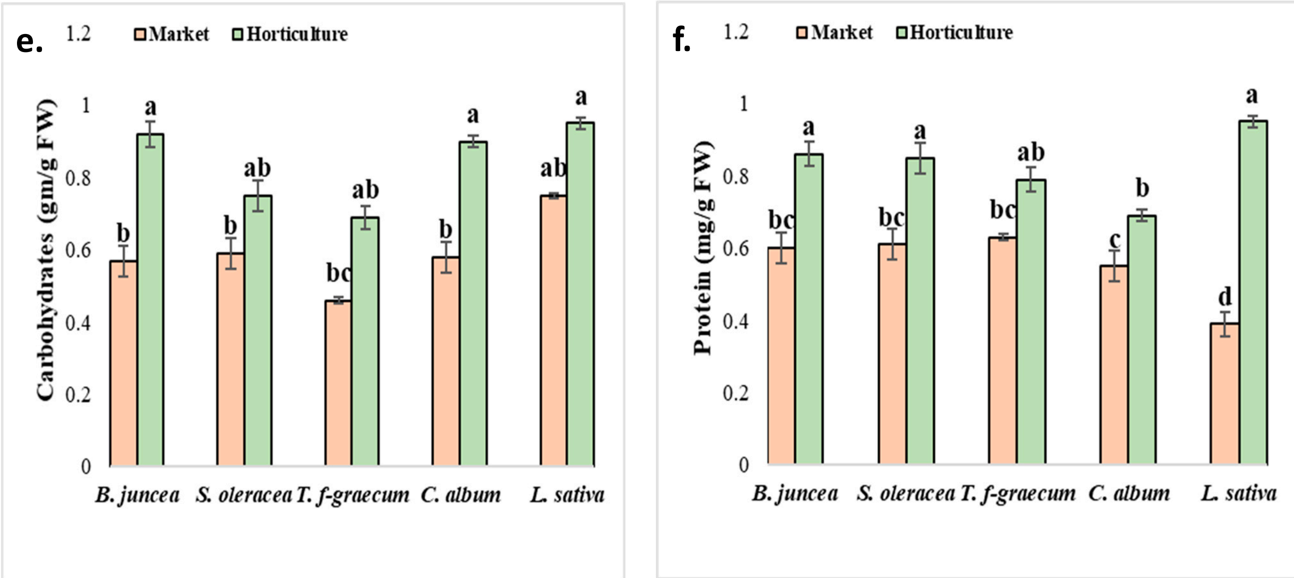


Figure 4. Moisture (**a**), Ash (**b**), Fats (**c**), Fibers (**d**), Carbohydrates (**e**) and Proteins (**f**) in leafy vegetables (*Brassica juncea*, *Spinacia oleracea*, *Trigonella foenum-graecum*, *Chenopodium album* and *Lactuca sativa*) collected from two different sites. Mean ± SD, $n = 3$. One-way ANOVA was performed, and the mean difference was tested by ($p < 0.05$). Letters on error bars illustrate significant difference between parameters of different vegetables.

3.3. Mineral Nutrients

The mineral nutrients illustrated significant variation among the selected leafy vegetables collected from the horticulture garden and the market (Figure 5). The vegetables from the horticulture garden depicted higher accumulations of Ca^{2+}, K^{+} and P^{+} contents, while the market vegetables showed greater values of Na^{+} content. Na^{+} content ranged from 33 to 77 ppm in *Brassica juncea* from the horticulture garden and *Spinacia oleracea* from the market, respectively. The Ca^{2+} content was the highest in *Chenopodium album*, while its lowest quantity was observed in *Lactuca sativa.* The maximum and minimum values of K^{+} were recorded in *Spinacia oleracea* and *Chenopodium album*, respectively. The cardinal limits for P^{+} were recorded in *Brassica juncea* from the horticulture garden and *Lactuca sativa* from the market.

3.4. Oxalate, Vitamin A and Tannins

The soluble oxalate exhibited greater variation among the leafy vegetables while the total oxalate showed almost similar behavior (Figure 6). Vegetables from the horticulture garden possessed higher vitamin A, soluble and total oxalate contents compared with the vegetables collected from the market. A higher accumulation of tannins was noticed in the vegetables collected from the market. The soluble oxalate varied from 0.7 to 2.86% in *Lactuca sativa* from the market and *Chenopodium album* from the horticulture garden, respectively. The upper and lower limits for the total oxalates were recorded in *Chenopodium album* and *Lactuca sativa.* The maximum value (1.18 mg g^{-1}f.w.) of vitamin A was noted in *Chenopodium album* and the minimum value (0.37 mg g^{-1}f.w.) in *Lactuca sativa. Brassica juncea*, *Spinacia oleracea* and *Chenopodium album* from the market were observed as having a higher quantity of tannins, while *Lactuca sativa* from the horticulture garden depicted the minimum concentration of tannins.

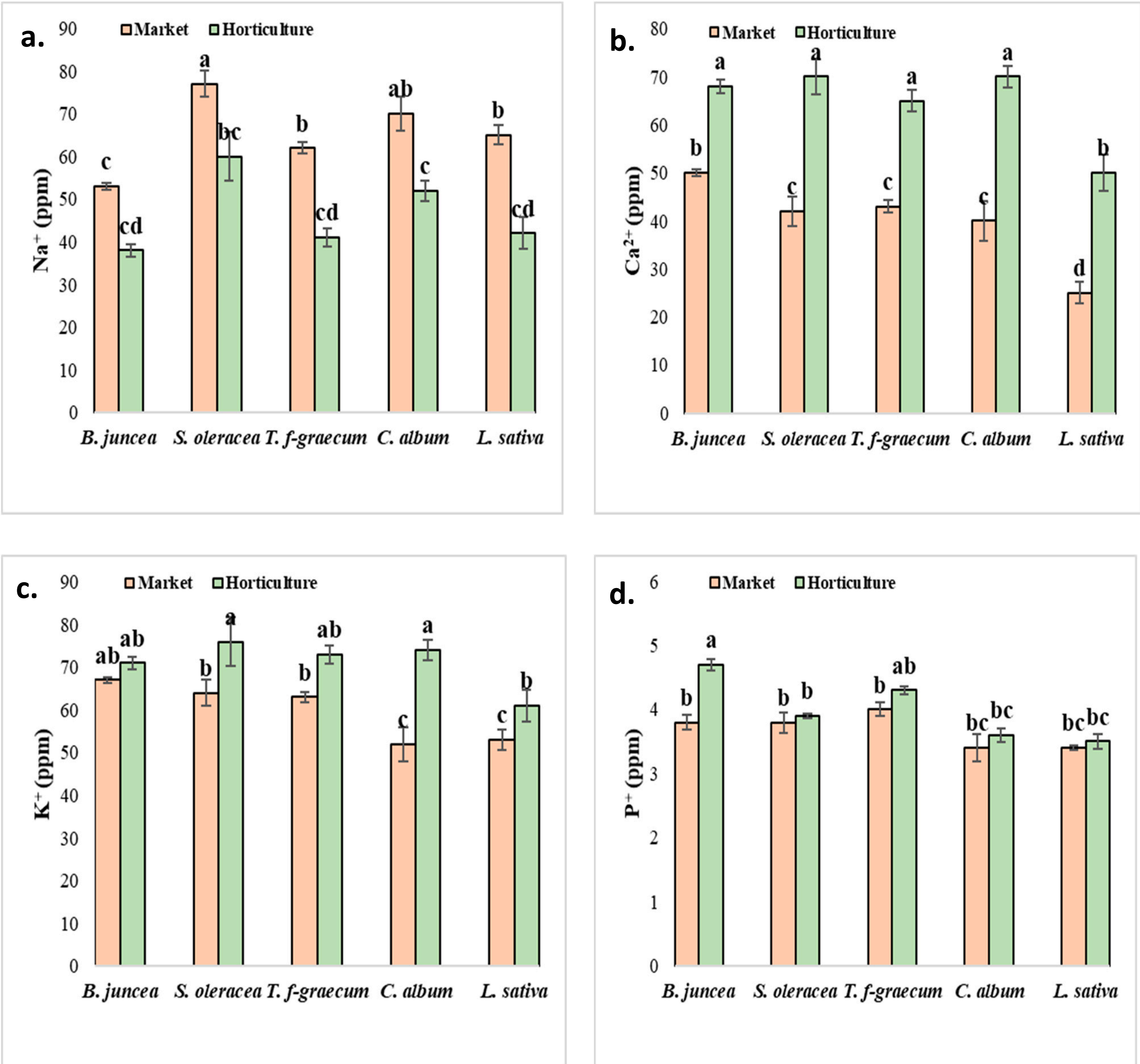


Figure 5. Na^+ (**a**), Ca^{2+} (**b**), K^+ (**c**) and P^+ (**d**) contents in leafy vegetables (*Brassica juncea*, *Spinacia oleracea*, *Trigonella foenum-graecum*, *Chenopodium album* and *Lactuca sativa*) collected from two different sites. Mean ± SD, $n = 3$. One-way ANOVA was performed, and the mean difference was tested by ($p < 0.05$). Letters on error bars illustrate significant difference between parameters of different vegetables.

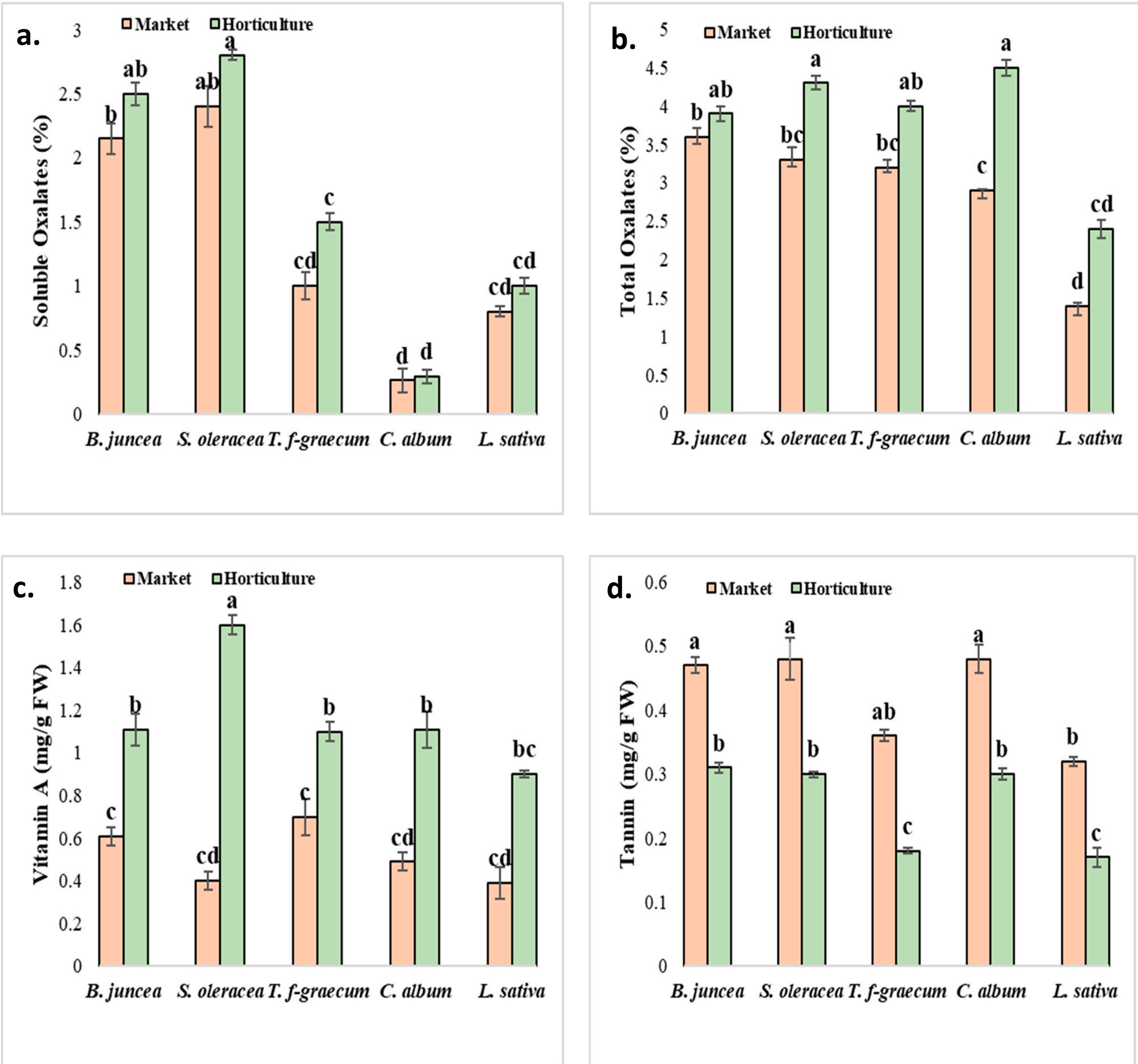


Figure 6. Soluble oxalate (**a**), Total oxalate (**b**), Vitamin A (**c**) and Tannins (**d**) in leafy vegetables (*Brassica juncea*, *Spinacia oleracea*, *Trigonella foenum-graecum*, *Chenopodium album* and *Lactuca sativa*) collected from two different sites. Mean ± SD, $n = 3$. One-way ANOVA was performed, and the mean difference was tested by ($p < 0.05$). Letters on error bars illustrate significant difference between parameters of different vegetables.

3.5. Multivariate Analysis

The principal component analysis (PCA) exhibited a significant association as well as variability among the physiological and nutritional attributes of the leafy vegetables (Figure 7). This PCA biplot showed 42.4% and 26.8% variation among the studied traits in Dim1 and Dim 2, respectively. The major contributors of Dim 1 are ash, proteins, carbohydrates, vitamin A, total oxalate, chlorophyll a, chlorophyll b, carotenes, Ca^{2+} and K^{+}, while Dim 2 comprised of tannins, fats and Na^{+}.

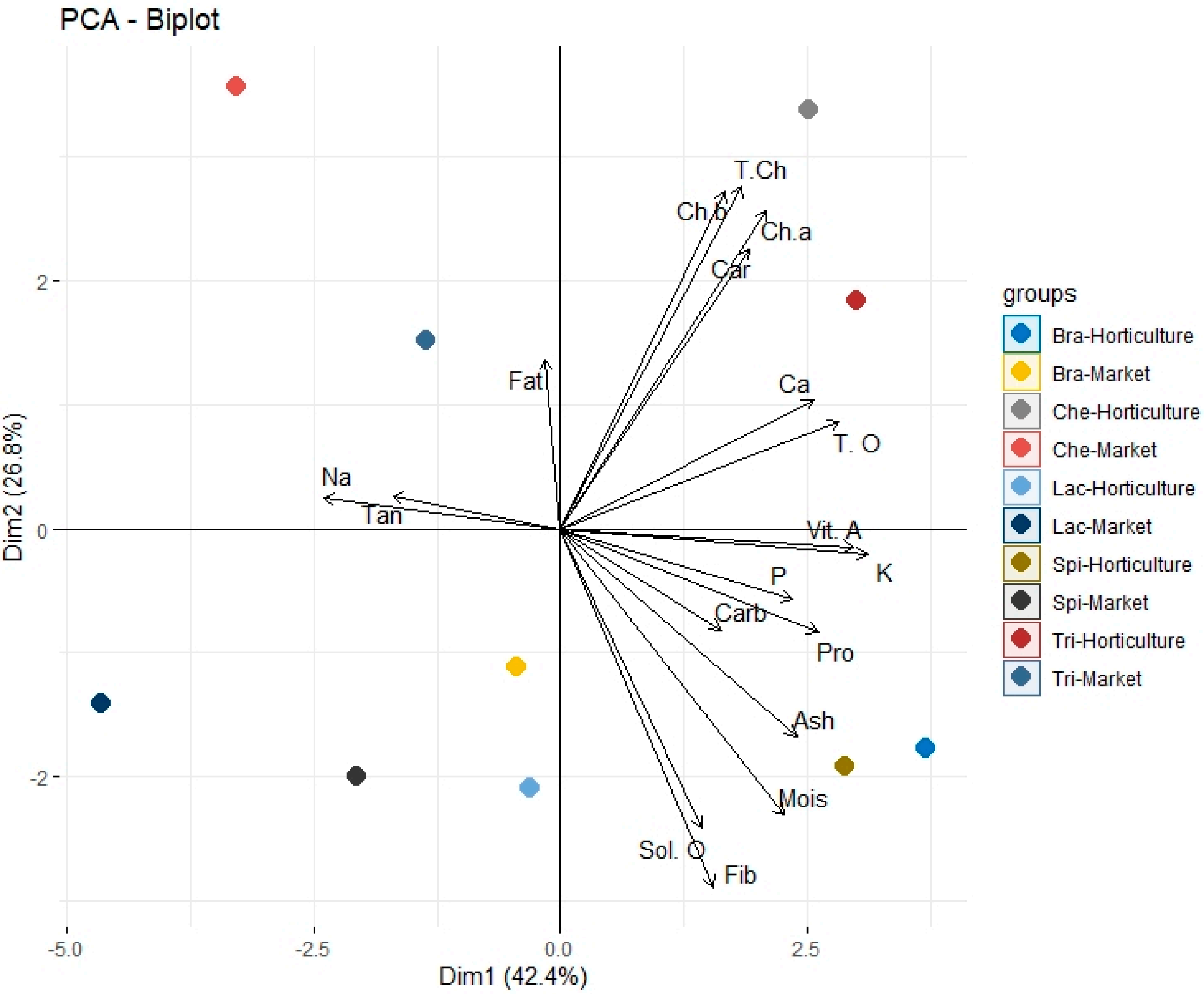


Figure 7. PCA biplot analysis among different physiological and nutritional attributes of leafy vegetables (Ch.a: Chlorophyll a, Ch.b: Chlorophyll B, T.Ch: Total chlorophyll, Car: Carotene, Mois: Moisture, Fib: Fibers, Carb: Carbohydrates, Pro: Proteins, Na: Na^+, Ca: Ca^{2+}, K: K^+, P: P^+, Sol.O: Soluble oxalate, T.O: Total oxalate, Vit.A: Vitamin A, Tan: Tannins).

3.6. Correlation Matrix

The correlation analysis examined the positive and negative relationship between physiological and nutritional attributes of leafy vegetables (Figure 8). Chlorophyll b is positively correlated with chlorophyll a, total chlorophyll and carotenes. Ca^{2+} possessed positive correlations with K^+ and total oxalates. Ash responded significantly to moisture, soluble oxalate and fibers. Protein depicted a negative relationship with Na^+, and carbohydrates showed a negative relationship with tannins.

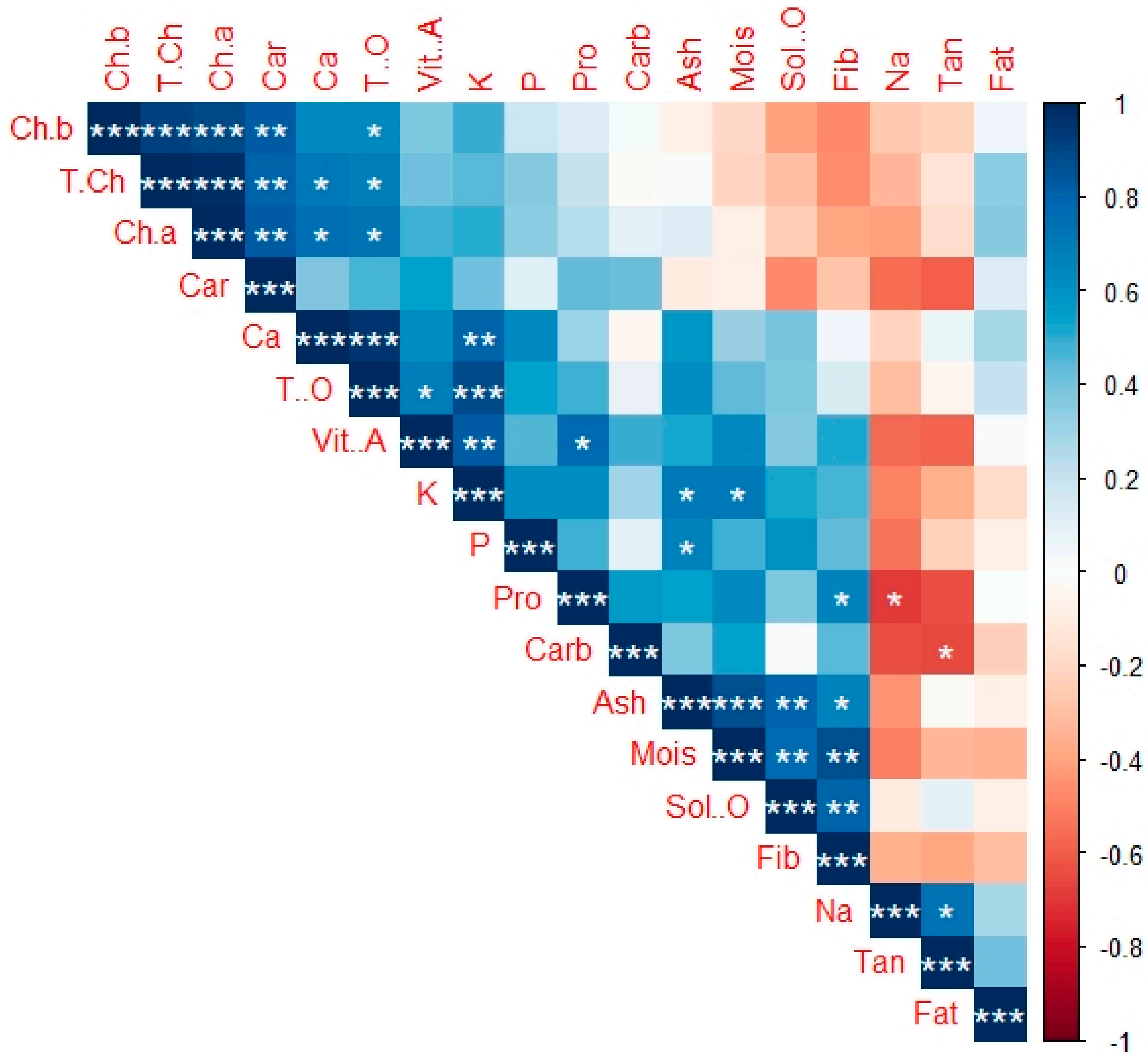


Figure 8. Correlation among different physiological and nutritional attributes of leafy vegetables (Ch.a: Chlorophyll a, Ch.b: Chlorophyll B, T.Ch: Total chlorophyll, Car: Carotene, Mois: Moisture, Fib: Fibers, Carb: Carbohydrates, Pro: Proteins, Na: Na^{+}, Ca: Ca^{2+}, K: K^{+}, P: P^{+}, Sol.O: Soluble oxalate, T.O: Total oxalate, Vit.A: Vitamin A, Tan: Tannins).

4. Discussion

Vegetables are quick sources of energy and are highly beneficial for the normal growth and development of the human body [11,12,31,32]. Vegetables possess essential chlorophyll contents, micronutrients, vitamins, proximates and oxalates [31]. However, vegetables sold in markets are expensive, low quality, stale and contain low micronutrients and minerals while also possessing high contents of tannins, which are toxic for human health [32,33]. Furthermore, the vegetables of the market have high concentrations of heavy metals (copper, cadmium, arsenic, mercury, lead and zinc), which are extremely toxic for human health and may even cause death [34,35]. In developing countries such as Pakistan, heavy metals in market vegetables are due to industrial emission, sewage waste and metal-containing fertilizers [36].

Chlorophyll is a green color pigment and usually present in two form, i.e., chlorophyll a and chlorophyll b [37,38]. Carotenoid is also a natural pigment found abundantly in almost all vegetables [39]. *Chenopodium album* from the horticulture garden possessed higher concentrations of chlorophyll and carotenoids than the other leafy vegetables explored in this experiment, and similar results were illustrated by [40]. In the present study, the market vegetables exhibited a decline in chlorophyll a, chlorophyll b, total chlorophyll and carotenoids, while vegetables from the horticulture garden showed greater chlorophyll and carotenoid contents. The variation in chlorophyll contents within the same species of different sites might be due to different harvesting times [40,41]. A difference in the growth conditions of two different sites may cause the redistribution of chloroplasts among mesophyll cells [42]. Similar results were found by [40] that indicate the level of chlorophyll and carotenoids depend upon species, varieties, cultivars, production practice, maturity and ecological factors such as light, water, temperature and soil properties.

In the current study, the leafy vegetables from the horticulture garden possessed more proximate contents than the vegetables collected from the market. Almost all the leafy vegetables of the experiment from the two sites contained higher amounts of proximate matter such as moisture, ash, fats, fibers, carbohydrates and proteins according to the work of [43]. *Lactusa sativa* is reported with elevated levels of moisture, fibre, carbohydrates and proteins in the horticulture garden, which are important for human health and should be provided in abundance for the normal growth and development of the human body [3,12,43]. Higher moisture contents in food helps people to digest it and assist bacterial action to act upon it [44]. Greater fat contents observed in *Spinacia oleracea* may prove sufficient for tissue repair, regulation of body processes and the biosynthesis of important enzymes and hormones [32,34,35,41]. Carbohydrates and proteins that play important roles in metabolic pathways were found at the highest concentrations in *Brassica juncea* and *Lactusa sativa* of the horticulture garden. Similar results were illustrated by [31,33].

Leafy vegetables are the sole source of micro-elements, as observed by the current study conducted on vegetables of two sites. The Ca^{2+}, K^{+} and P^{+} contents were observed to be at the lowest in vegetables from the market, while higher concentrations of Na^{+} were reported in market vegetables. *Spinacia oleracea* from the horticulture garden exhibited a sufficient quantity of K^{+}, which may help to improve water retention and prevent osteoporosis and kidney stones [36]. The contents of P^{+} were found to be abundant in *Brassica juncea,* which is reported to assist in making proteins for growth, maintenance and repairing of tissues [12,18,36]. Healthy vegetables such as those reported in the horticulture garden can provide all these elements and improve quality of life [1,43,45]. *Spinacia oleracea* collected from the market illustrated elevated level of Na^{+}, which is not required from vegetables because it is already available in human body excessively due to higher uptake of NaCl [10]. The minimum quantity of Na^{+} and a higher level of K^{+} in the diet can contribute to decreased cardiovascular diseases in human [12]. *Spinacia oleracea* and *Chenopodium album* from the horticulture garden were observed with sufficient quantities of Ca^{+2}, which plays an important role in human health as plant-based foods provide 25% of the total dietary calcium to humans [6].

Oxalates are very important anti-nutrients found in excessive quantities in *Chenopodium album* collected from the horticulture garden, which contain important antioxidants and fibers required for the normal growth and development [43,46,47]. *Brassica juncea* and *Chenopodium album* collected from the horticulture garden illustrated increased vitamin A contents, which prove essential for the maintenance of the immune system and efficient functioning of the retina [10,23,41,42]. Greater amounts of tannins were seen in *Spinacia oleracea* and *Chenopodium album* collected from the market, which can impart some side effects such as skin rash, stomach irritation, nausea, vomiting, liver damage and kidney failure [20,48]. Furthermore, high concentrations of tannins in plants can reduce the crop yield in different species [49]. Our findings revealed that the vegetables from the horticulture garden have significant amounts of oxalates and vitamins, while reduced concentration of tannins, ultimately beneficial for the normal growth and development of the human body.

5. Conclusions

This research illustrated the significance of plant species, especially leafy vegetables used in the daily diet. The significance of these leafy vegetables was chiefly related to their photosynthetic pigments, proximate matter and nutrients. This study includes five leafy vegetables collected from two sites (a horticulture garden and a market) with environmental differences. Physiological and nutritional analyses were conducted to demonstrate the nutrient composition of the selected leafy vegetables. Among the five selected vegetables, *Trigonella foenum-graecum* was the poorest in nutrition both in the horticulture garden and the market. *Brassica juncea* was the best sources of vitamin A, Ca^{2+} and carbohydrates, while *Lactuca sativa* leaves were the best source of fibre and proteins. Almost all these leafy vegetables exhibit great contributions towards deitary requirements, but *Brassica juncea* and *Lactuca sativa* surpassed all the other vegetables regarding nutrition. Due to this reason, the consumption and cultivation of these two leafy vegetables must be promoted. It is also revealed that vegetables from the horticulture garden are fresher, more cost-effective and more nutrient-rich than those from the market. However, a wide range of research is required to analyze the quantification of minerals, antioxidants, vitamins, heavy metals and anti-nutrients in vegetables. Furthermore, some pharmacological analysis should be performed in future for ethnomedicinal attributes.

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