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Impact Of Emerging Farming Practices On Antioxidant Activity In Commonly Eaten Leafy Vegetables

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Abstract
Antioxidants are essential compounds that neutralize free radicals, reduce oxidative stress, and help prevent chronic diseases. Among all vegetables, leafy greens are an excellent source of antioxidants. Sustainable farming techniques, such as organic and hydroponic farming, are practiced to enhance the nutritional content of vegetables at the farm level. These methods can significantly influence their antioxidant levels. The study was designed to compare antioxidant activity in leafy greens grown using conventional, organic, and hydroponic farming methods and to evaluate the potential of organic and hydroponic farming as strategies for improving dietary antioxidant intake. DPPH (2,2-diphenyl-1-picrylhydrazyl) assay was used to measure antioxidant activity. Samples were procured from conventional, organic, and hydroponic vendors, analyzed for their antioxidant activity, and the results were statistically compared using ANOVA. Organic and hydroponic methods showed significantly higher antioxidant activity compared to conventional farming. Fenugreek and coriander grown organically exhibited the highest antioxidant levels, while mint displayed the highest antioxidant activity under hydroponic conditions. The three farming methods had significant differences ($p<0.05$). This study demonstrates that organic and hydroponic farming practices can increase the antioxidant content in leafy greens, resulting in higher nutritional value.
Keywords: *Antioxidants, conventional, DPPH assay, hydroponic, leafy vegetables, organic*

INTRODUCTION

Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and antioxidant defences, has been widely recognized as a central mechanism in the pathophysiology of chronic and degenerative illnesses, such as diabetes mellitus, cancer, cardiovascular disorders, and neurodegenerative conditions [1]. Free radicals such as ROS, containing one or more unpaired electrons in the outermost orbital, are the most unstable species that exists independently, causing oxidative stress [2]. Oxidative stress is a worldwide phenomenon characterized by an imbalance in the number of pro-oxidants and antioxidants in favor of pro-oxidants [3]. The human body can protect itself from these free radicals by scavenging ROS and producing endogenous or exogenous antioxidant compounds that scavenge free radicals [4,5].

Antioxidants donate electrons to free radicals, stabilizing them and stopping the chain reaction of damage, therefore, preventing oxidative damage to cellular components and biological compounds (such as proteins, lipids, or other compounds), and thereby providing a protective effect against ROS (such as superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen, and peroxyl radical) [6]. Antioxidant nutrients are present in the diet in the form of (poly)phenols, flavonoids, carotenoids, vitamin E, and ascorbic acid. Fruits and vegetables serve as the primary dietary sources of these compounds, particularly leafy greens [7]. Leafy greens are widely acknowledged for their high antioxidant properties, mainly due to the presence of bioactive compounds such as polyphenols, flavonoids, carotenoids, and tocopherols, that work against oxidative damage to cellular components, thereby minimizing the incidence of chronic diseases [8]. In addition, the antioxidant potential varies with crop type and cultivation practices.

Numerous studies have shown variations in the antioxidant activity of leafy greens. For instance, in Assam, the antioxidant activity of selected GLVs ranged from 18.64% to 69.34%. *Hibiscus sabdariffa* exhibited the highest inhibition (69.34%), while *Spinacia oleracea* showed the lowest (18.64%) [9]. Suresh et al. [10] reported sirukeerai (98.42%), mint (96.85%), and spinach (96.06%) with the highest antioxidant activity among the ten GLVs studied. Indigenous leafy greens are rich in antioxidants and are helpful for the treatment of chronic ailments [11]. Generally, leafy greens outperform other vegetables in antioxidant value [12]. The antioxidant activity of leafy greens is often higher than that of root vegetables [8,13]. These variations in antioxidant activity highlight not only the influence of plant species but also raise questions about the role of farming practices on antioxidant properties.

With the growing advancements in farming methods, particularly organic and hydroponic farming, there is increasing interest in understanding how these methods influence the antioxidant properties of

vegetables. Organic farming is a soil-based method that relies on natural resources and inputs for production, while hydroponic farming is a soil-less system that uses nutrient-enriched water solutions [14,15]. While both methods offer potential advantages, their impact on the antioxidant activity of leafy vegetables remains inconclusive, underscoring the need for systematic evaluation.
Screening of antioxidants in leafy greens requires appropriate methods, which address the mechanism of antioxidant activity and focus on the kinetics of the reactions including the antioxidants [1]. Studies focusing on antioxidant activity in various types of samples are using different methods. Among them, 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulphonate) radical (ABTS·+) scavenging, 1,1-diphenyl-2-picrylhydrazyl (DPPH·) radical scavenging, and ferric reducing antioxidant power (FRAP) assay are widely employed [2]. Out of these, DPPH radical scavenging assay is the most commonly used method for evaluating antioxidant activity in fruits and vegetables due to its operational simplicity, reproducibility, and sensitivity in assessing radical scavenging efficiency [16]. DPPH radical is a stable nitrogen-centered radical with a deep violet color and a strong absorption maximum at 517 nm. When an antioxidant donates a hydrogen atom or electron, the radical is reduced, causing a measurable decline in absorbance. The degree of discoloration corresponds to the scavenging ability of the test sample and is expressed as percentage inhibition (% inhibition), with higher values reflecting greater antioxidant potential [17].
Therefore, the present study used the DPPH radical scavenging assay method to evaluate the antioxidant activity of selected leafy greens grown using conventional, organic, and hydroponic farming methods. The specific objectives were: (i) to compare antioxidant activity in selected leafy greens grown under conventional, organic, and hydroponic farming practices, and (ii) to assess the potential of organic and hydroponic methods in enhancing antioxidant levels in commonly consumed leafy greens.

MATERIALS AND METHODS

Coriander (*Coriandrum sativum*), fenugreek (*Trigonella foenum-graecum*), and mint (*Mentha spicata*) leaves were selected for the study. Conventionally grown leafy greens were purchased from the local market. The same variety of the three selected samples was procured from locally available organic and hydroponic vendors. Each sample was collected in triplicate. The samples were taken to the laboratory, and the testing was done on the same day of sample collection. The chemicals and reagents used in the testing were of analytical grade.
For sample extraction, 0.5 g of the homogenized sample was mixed with 5 mL of ethanol, and 0.5 mL of this extract was combined with 3 mL of ethanol and 0.3 mL of ethanolic 0.5 mM DPPH (CAS Number: 1898-66-4, Sigma-Aldrich, Inc., United States) solution. A blank was prepared with 0.5 mL sample and 3.2 mL ethanol (no DPPH), and a control with 3.5 mL ethanol and 0.3 mL DPPH (no sample). L-ascorbic acid (CAS Number: 50-81-7, Sigma-Aldrich, Inc., United States) was used as a reference. All mixtures were kept in the dark at room temperature for 30 min, and absorbance was measured at 517 nm using a UV-Vis spectrophotometer (Evolution 201, Thermo Scientific, US) [18]. The results were calculated using this formula:

$$Antioxidant\ activity\ (\%\ Inhibition) = 100 - \{\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control}\} X\ 100$$

The results were statistically analyzed using one-way Analysis of Variance (ANOVA) in SPSS (Statistical Package for the Social Sciences, Version 20.0; IBM Corp., Armonk, NY, USA). The level of statistical significance was set at $p < 0.05$. If significant differences were observed, a post-hoc multiple comparison test (Tukey's HSD) was performed to determine pairwise group differences.

RESULTS

Figure 1 presents the antioxidant activity (%) of coriander, fenugreek, and mint leaves procured from conventional, organic, and hydroponic vendors. A clear variation was observed among the three farming methods.

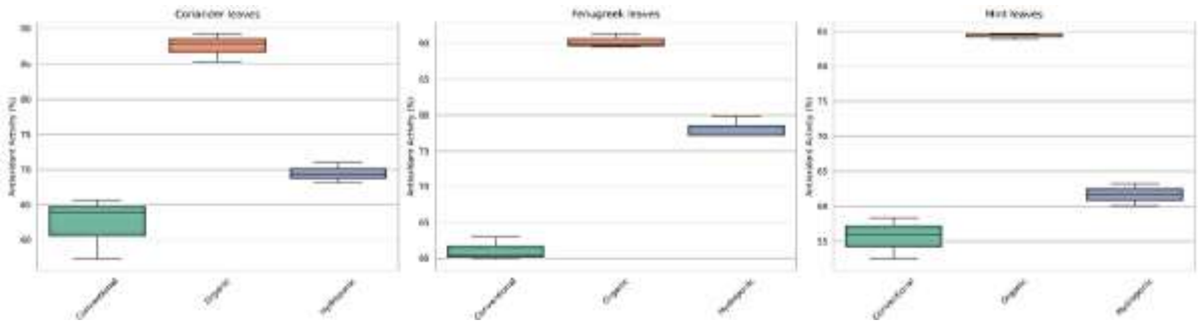

Figure 1: Antioxidant activity (%) in different leafy greens and farming methods (a) coriander leaves, (b) fenugreek leaves and (c) mint leaves

In addition, table 1 shows the ANOVA results for the leafy greens. For coriander leaves, organic method (87.51±2.00%) showed higher antioxidant activity compared to hydroponic (69.50±1.39%) and conventional (62.28±4.19%) ones, with ANOVA indicating a significant effect (F statistics = 53.17). Similar patterns were observed for fenugreek leaves, where organic method (90.29±0.92%) exhibited higher antioxidant activity than hydroponic (78.08±1.33%) and conventional (61.04±1.35%), supported by ANOVA significance (F statistics = 76.25). For mint leaves, antioxidant activity was highest in organic (84.39±0.85%), followed by hydroponic (61.65±1.54%) and conventional (55.56±2.97%), with ANOVA showing a strong effect (F statistics = 162.34, $p < 0.001$). Tukey's HSD test confirmed significant differences between organic and both conventional and hydroponic samples across all three leafy greens ($p < 0.001$). While hydroponic methods showed moderately higher activity than conventional ones in fenugreek ($p < 0.001$) and mint ($p = 0.0213$), the difference for coriander was not statistically significant ($p = 0.0534$).

Table 1: Comparison of the antioxidant activity of leafy greens grown using different farming methods using ANOVA

Leafy Greens	Farming Method	Antioxidant Activity (% Inhibition)	AVOVA	p-value
		Mean±SD	F-statistics value	
Coriander leaves	Conventional	62.28±4.19^{b}	53.17	<0.001
	Organic	87.51±2.00^{a}		
	Hydroponic	69.50±1.39^{b}		
Fenugreek leaves	Conventional	61.04±1.35^{c}	76.25	<0.001
	Organic	90.29±0.92^{a}		
	Hydroponic	78.08±1.33^{b}		
Mint leaves	Conventional	55.56±2.97^{c}	162.34	<0.001
	Organic	84.39±0.85^{a}		
	Hydroponic	61.65±1.54^{b}		

DISCUSSION

The present study showed significant variation in antioxidant activity among the leafy greens (coriander, fenugreek and mint leaves) grown under different farming practices. Organic farming consistently yielded the highest antioxidant activity among the farming methods, with fenugreek leaves showing the maximum inhibition (90.29%), followed by coriander (87.51%) and mint (84.39%). A higher percentage inhibition reflects greater antioxidant capacity, indicating the ability of the sample to counteract oxidative stress. Such results coincide with the results reported by Malešević et al. [19] and de Oliveira Pereira et al. [20], who found higher antioxidant activity in organically grown leafy greens compared to conventionally grown ones. Other organically grown leafy greens such as arugula, chicory and lettuce also showed significantly greater DPPH inhibition when compared to their conventional samples [21,22]. The enhanced antioxidant activity in organic methods could be due to the absence of synthetic fertilizers and

reliance on organic manures and biostimulants that stress plants in a way that stimulates secondary metabolites like phenolics and flavonoids, thereby increasing antioxidant activity [23,24].
Hydroponically grown leafy greens consistently exhibited intermediate antioxidant levels, higher than conventional but lower than organic. The absence of soil-borne pathogens in hydroponics reduces stress on plants, allowing for better nutrient absorption and higher antioxidant levels [25,26]. Conversely, while hydroponic systems show advantages in antioxidant activity, organic methods may still yield vegetables with higher antioxidant properties, particularly when organic fertilizers are used [23,27].

CONCLUSION

The study concludes that organic framing practice confers the most significant advantage in enhancing the antioxidant activity of leafy greens. At the same time, hydroponics represents a promising alternative to conventional methods, producing moderately improved outcomes. This shows that the emerging new farming practices are not only helpful in managing resources but also in improving the nutritional properties of leafy greens.

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