Original Research

International Journal of Contemporary Microbiology/Volume 10 No. 1, 2024

Patterns of Phytochemical Variation in Populations of Amaranthus Viridis In Two Ecological Zones Of Ghana

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How to cite this article: Antwi G, Essilfie MK. Patterns of Phytochemical Variation in Populations of Amaranthus Viridis in Two Ecological Zones of Ghana.International Journal of Contemporary Microbiology/ Volume 10 No. 1, 2024

ABSTRACT

The study aimed to assess the patterns of variations in total phenolic content, total flavonoid content, and antioxidant activity in populations of *A. viridis* in two different ecological zones of Ghana. Matured plant leaves of Amaranthus viridis were collected at two different locations namely Aburi Botanical Gardens and Legon Botani-cal Gardens and were subjected to ethanolic extraction. The extracts were analyzed for antioxidant activity using 1,1-diphenyl 1,1-2 picrylhydrazyl radical scavenging, total phenolic content was analyzed using Folin-Ciocalteu reagent, and total flavonoid content was also analyzed using the Aluminium chloride colorimetric technique. The correlation of total phenolic content and total flavonoid content, total phenolic content and antioxidant activity, and, total flavonoid content and antioxidant activity all yielded a directly proportional association. Thus, as total phenolic content increased total flavonoid content also increased and this applies to all the scenarios above. There was a substantial difference in the phenolic content as Aburi Botanical Gardens had a higher phe-nolic content than Legon Botanical Gardens. The semi-deciduous forest of the Aburi Botanical Garden and the coastal savannah of the Legon Botanical Garden, two contrasting ecological contexts, have diverse effects on the phytochemical composition of Amaranthus viridis, as this study has clarified.

Keywords: Amaranthus viridis, total phenolic content, total flavonoid content, antioxidant activity, ecological zones, Ghana

INTRODUCTION

Amaranthus viridis is a vigorous annual or short-lived perennial herb that belongs to the family Amaranthaceae. The leaves are eaten occasionally as cooked vegetables or as a replacement for spinach. The leaves are diuretic and purgative and are widely used in the pharmaceutical industry to produce medicinal products that possess antifungal, anti-inflammatory, and antiseptic qualities. *Amaranthus viridis* is used as traditional medicine in the treatment of fever, pain, asthma, diabetes, dysentery, urinary disorders, liver disorders, eye disorders, and venereal diseases 1. The plant also possesses anti-microbial properties. The leaves are diuretic and purgative and used as poultices (fresh or as dried powder) to treat inflammations, boils, gonorrhoea, haemorrhoids, and abscesses.

In Africa, traditional medicine plays a very important role in the health system. The medicinal abilities of certain plants are determined by the presence of bioactive substances produced in the plants. According to [2], the phytochemical screening of *Amaranthus viridis* revealed that *A. viridis* is highly rich in phenols such as catechins, protocatechuic acid, and rutin. Also, flavonoid compounds such as quercetin, luteolin, and kaempferol which are known for their antioxidant activity are found in *Amaranthus viridis*. Other

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phytochemicals such as vitamin C, vitamin B, and carotenoids apart from phenols, flavonoids, and antioxidants can be found in *Amaranthus viridis.* Phenols present in plants contain aromatic ring with one or more hydroxyl groups which acts as antioxidants and hence enable the plant to eliminate free radicals introduced into the plants. Biologically active compounds that are found in the same plant species may be different in content and quantity because of the environmental differences in their growing area [3]. Bioactive substances are often the outcome of the interaction between plants and their environment. These substances can be influenced by various environmental factors such as soil composition, climate, sunlight exposure, and interactions with other organisms like pollinators and microbes. Certain phytochemicals can only be synthesized under specific environmental conditions. For example, the phenolic acid caffeic acid may increase in plants exposed to UV light as a protective response.

Despite that, there have been several studies that have been conducted on the medicinal and phytochemical properties of *A. viridis*, but there are still few studies comparing different populations of Amaranthus viridis in Ghana. Understanding the differences between these populations can prove useful for harvesting this plant species for the production of herbal medicines, adding its natural antioxidants to the diet, and improving food preservation techniques. Ghana comprises six agroecological zones, each characterized by distinct rainfall patterns and soil types [4]**.** According to [5], rainfall patterns range from 780 mm to 2,200 mm annually, with uni-modal patterns in the Coastal, Sudan, and Guinea Savannah zones, and bi-modal patterns in the remaining zones. Soils in the Forest zone are predominantly acrisols and lixisols, deep and easily tilled, but with low nitrogen and phosphorus content. In contrast, soils in the Guinea and Sudan savannah zones are shallower, with lower organic carbon and nitrogen levels, posing challenges for agriculture compared to the more fertile Forest Zone. [5]

The study on *Amaranthus viridis* will involve a thorough process to identify patterns of variation in phytochemical content, including total phenols, total flavonoids, and antioxidant activity. Initially, phytochemicals will be extracted from *Amaranthus viridis* leaves using 100% ethanol. After 24 hours, the filtrates will be obtained and stored for analysis. Total phenolic content will be determined using the Folin-Ciocalteu method, while total flavonoid content will be assessed using a modified aluminum chloride colorimetric method. Antioxidant activity will be evaluated using the DPPH assay. These analyses aim to uncover differences in phytochemical composition across various locations where *Amaranthus viridis* is found.

MATERIALS AND METHODS

Description of Study Area

The study area encompasses two distinct regions: Aburi Botanical Gardens and Legon Botanical Gardens. Aburi Botanical Gardens, situated on the Akwapim ridge approximately 38 kilometers northeast of Accra, falls within the Tropical Rainforest Zone. This location experiences a bimodal precipitation pattern, with primary rainfall from May to June and secondary rainfall from September to November. The average annual rainfall is 1270mm, with an average temperature of 23.88°C. The predominant flora consists of semi-deciduous woodland. Similarly, Legon Botanical Garden, also within the Tropical Rainforest Zone, exhibits a tropical monsoon climate characterized by well-defined wet and dry seasons. The wet season typically extends from April to October, with substantial rainfall, while the dry season occurs from November to March. Average temperatures range from 28 to 32°C, with relatively high humidity levels during the wet season.

Materials

Leaves of *Amaranthus viridis*, electric balance, McCartney bottles, Micropipette, funnel, conical flasks, UV spectrophotometer, Whatman filter paper, graduated measuring, cylinder, test tubes, cuvettes, vortex mixer, oven.

The reagent used was 1, 1-diphenyl-2-picrylhydrazyl (DPPH), Rutin, and Folin- Ciocalteu reagent. The chemicals that were also used were sodium carbonate (100g), ethanol, distilled water, sodium carbonate (Na_2CO_3), sodium hydroxide (($NaOH$), and aluminum chloride ($AlCl3. H_2O$), sodium nitrite ($NaNO_2$).

Collection of Plant Samples

A total of twenty (20) in1dividual plant samples of Amaranthus viridis were gathered from each of the two distinct locations in Ghana. Specifically targeted were samples that had flowered or were bearing fruit, which were then uprooted from the ground to ensure the entire plant form was obtained. Following collection, the samples were dried away from direct sunlight for a period of 2 weeks and subsequently ground into fine particles before being sent for extraction (Table 1).

Extraction of phytochemicals in Amaranthus viridis

A mass of 5.0g of all the grounded leaves for all the individual *Amaranthus viridis* was measured using an electric balance and poured into McCartney bottles. Twenty milliliters (20ml) of 100% ethanol were added and shaken on a shaker for 4 hours. The bottles were covered and allowed to stand for 24 hours. After 24 hours, the sample in solution was first filtered through cotton wool, then through Whatman filter paper No.1 (125mm), and the filtrate was stored in tightly covered McCartney bottles at a temperature of 4°C in a fridge. The filtrates were used for the appraisal of the various phytochemical tests.

Test for total phenolic content

For each specific location, the analysis of phenolic compounds within the Amaranthus viridis extracts was carried out employing the Folin-Ciocalteu method as outlined [6]. Following a 24-hour duration, precisely 4 milliliters of each extract were measured using a graduated cylinder. Subsequently, these measured extracts were diluted to a volume of 20 milliliters using distilled water within individual test tubes. To prepare the samples for analysis, 40 milligrams per milliliter (mg/ml) of the diluted samples were carefully transferred into cuvettes.

The next steps involved precise measurements: 1.58 milliliters of distilled water and 100 milligrams per milliliter (mg/ml) of the Folin-Ciocalteu reagent were respectively measured using a graduated cylinder and a micropipette, and then added to the solution. To ensure thorough mixing, the solution was vigorously shaken using a vortex. After allowing the mixture to stand for 5 minutes, a volume of 300 milligrams per milliliter (mg/ml) of sodium carbonate was pipetted and introduced into the solution, followed by another round of shaking.

Table 1: Geographical coordinates of the *Amaranthus viridis* that were collected in Aburi Botanical Gardens and Legon Botanical Gardens.

LOCATION	PLANT SPECIES	No. of SAMPLES	LONGITUDE	LATITUDE	ALTITUDE	VEGETATION
ABURI	*Amaranthus viridis*	5	5° 51’ 17” N	0° 10’ 15” N	443m	Semi-deciduous forest
ABURI	*Amaranthus viridis*	4	5° 51’ 16” N	0° 10’ 18” N	449m	Semi-deciduous forest
ABURI	*Amaranthus viridis*	5	5° 51’ 19” N	0° 10’ 12” N	441m	Semi-deciduous forest
ABURI	*Amaranthus viridis*	6	5° 51’ 15” N	0° 10’ 13” N	437m	Semi-deciduous forest
LEGON	*Amaranthus viridis*	5	5° 39’ 40” N	0° 11’ 24” N	114m	Coastal Savannah
LEGON	*Amaranthus viridis*	3	5° 39’ 38” N	0° 11’ 22” N	111m	Coastal Savannah
LEGON	*Amaranthus viridis*	6	5° 39’ 37” N	0° 11’ 23” N	118m	Coastal Savannah
LEGON	*Amaranthus viridis*	2	5° 39’ 42” N	0° 11’ 21” N	113m	Coastal Savannah
LEGON	*Amaranthus viridis*	4	5° 39’ 43” N	0° 11’ 29” N	121m	Coastal Savannah

To initiate the chemical reaction, the solution was placed in an oven set at a temperature of 40°C for a duration of 30 minutes. Once this incubation period was completed, the cuvettes containing the solution were carefully removed from the oven and allowed to sit undisturbed for an additional 90 minutes. The measurement of absorbance at 765 nanometers (nm) was then determined, utilizing ethanol as the blank reference, with the assistance of a visible spectrophotometer.

Test for total flavonoid content

The determination of flavonoid content in the plant extract was conducted following the modified aluminum chloride colorimetric method as described by [7]. To initiate the process, a sample extract with a concentration of 100 milligrams per milliliter (mg/ml) was introduced into a solution containing 500 mg/ml of distilled water and 30 mg/ml of sodium nitrite ($NaNO_2$) at a concentration of 5%. This resulting mixture was allowed to stand undisturbed for a period of 5 minutes.

Following this, a solution composed of aluminum chloride ($AlCl3 \cdot H_2O$) at a concentration of 10% and 30 mg/ml was added to the mixture. Once again, the mixture was left to stand, this time for 6 minutes. To further process the solution, sodium hydroxide (NaOH) with a concentration of 1M and 200 mg/ml of distilled water was introduced into the mixture, and thorough mixing was achieved using a vortex.

To determine the flavonoid content quantitatively, measurements of the solution's absorbance were performed at a wavelength of 425 nanometers (nm) using a spectrophotometer. It's noteworthy that the flavonoid compound Rutin was employed as the reference standard for this analysis.

Test for antioxidant activity (DPPH)

The DPPH assay was conducted following the methodology outlined by [8], albeit with certain modifications. Initially, a solution of DPPH with a concentration of 0.1 millimoles per liter (mmol/L) was freshly prepared by dissolving DPPH in ethanol and subjecting it to ultrasonic bath homogenization for a duration of 30 seconds. Subsequently, 2 milliliters of the sample solution were vigorously mixed with an equal volume (2 milliliters) of the freshly prepared DPPH solution. This resulting mixture was then left to incubate in darkness at room temperature for a period of 30 minutes. Following the incubation period, the absorbance of the solution was determined at a wavelength of 517 nanometers (nm).

RESULTS

The study examined the variability of total phenols, total flavonoids, and antioxidant activities in A. viridis populations at two locations. Both locations showed slightly different mean values, minimum and maximum values, standard deviation, and standard errors, indicating less variability. Total flavonoids yielded the same results, but the standard deviation at Aburi was higher than Legon, indicating greater variability. The antioxidant activities showed a similar trend, but with a significant difference in standard error. Samples from Aburi Botanical Gardens had greater variability than those from Legon Botanical Gardens. The student t-test revealed significant differences in total phenolic content between Aburi and Legon, indicating a real and meaningful association between the two locations. No significant difference in total flavonoid content was found between Aburi and Legon, indicating a spread-out data set. The antioxidant activity variation was slightly higher in Aburi than Legon. Correlation graphs showed significant correlations between TPC and TFC, TFC and DPPH, and TPC and DPPH, with a positive correlation for all three cases (Plate 1).

Relationship of total phenolic content in both locations:

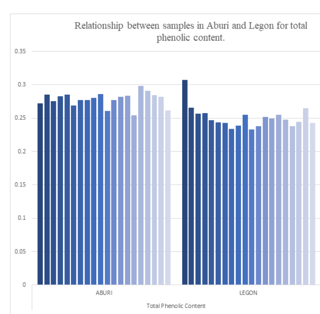

Fig. 1: A bar graph showing the relationship between the total phenolic content found in the two locations.

Plate 1: A photograph of *Amaranthus viridis* showing the inflorescence flowers in the field

Table 2: t-test of the phenolic contents between the two ecolog ical zones.

t-Test: Two-Sample Assuming Equal Variances		
Total Phenolic Content	**Aburi**	**Legon**
Mean	0.28	0.25
Variance	0.0001	0.0003
Observations	20	20
Pooled Variance	0.0002	
Hypothesized Mean Difference	0	
Df	38	
t Stat	6.3	
P(T<=t) one-tail	0.00000009	
t Critical one-tail	1.70	
P(T<=t) two-tail	0.0000002	
t Critical two-tail	2.02	

Relationship of total antioxidant activity in both locations:

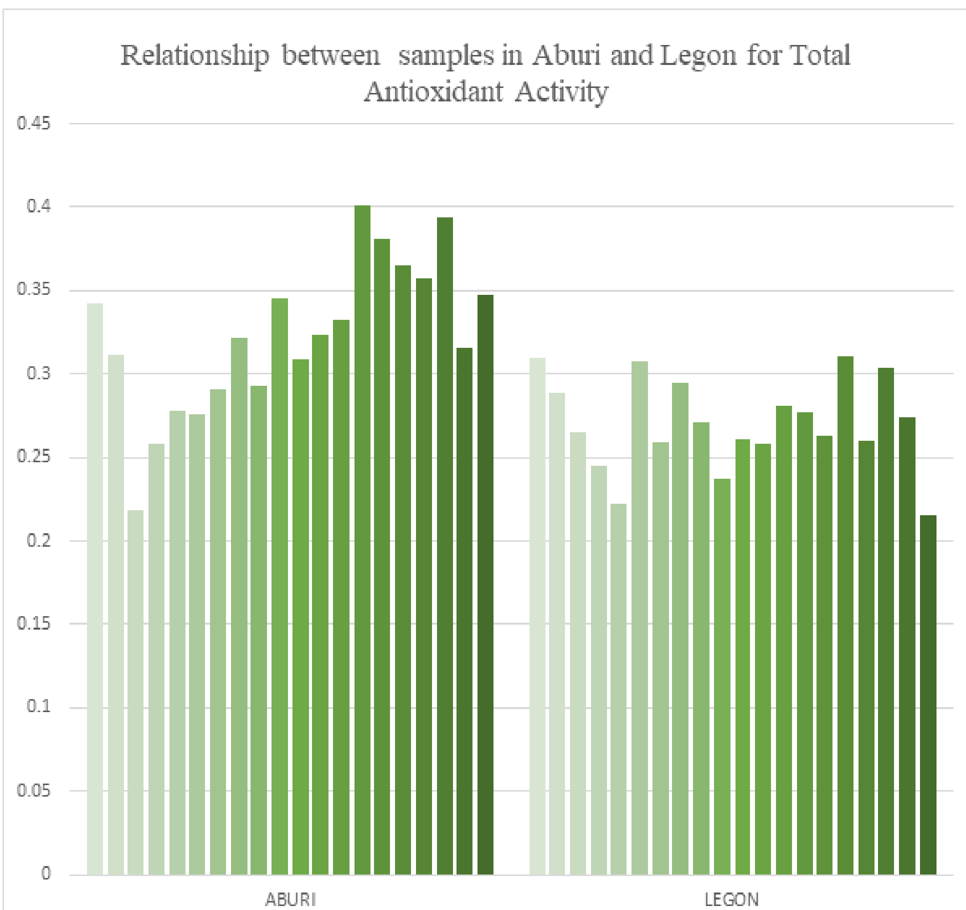

Figure 2: A bar graph showing the relationship between the total antioxidant activity found in the two locations.

Table 3: t-test of the total flavonoid contents between the two ecological zones

t-Test: Two-Sample Assuming Equal Variances		
Total Flavonoid content		
	Aburi	**Legon**
Mean	0.01	0.1
Variance	0.0003	0.00005
Observations	20	20
Pooled Variance	0.0002	
Hypothesized Mean Difference	0	
Df	38	
t Stat	-1.4	
P(T<=t) one-tail	0.10	
t Critical one-tail	1.70	
P(T<=t) two-tail	0.20	
t Critical two-tail	2.02	

Table 4: t-test of the antioxidant activity between the two ecological zones.

t-Test: Two-Sample Assuming Equal Variances		
Antioxidant activity		
	Aburi	**Legon**
Mean	0.32	0.27
Variance	0.002	0.008
Observations	20	20
Pooled Variance	0.002	
Hypothesized Mean Difference	0	
Df	38	
t Stat	4.38	
P(T<=t) one-tail	0.00005	
t Critical one-tail	1.70	
P(T<=t) two-tail	0.00009	
t Critical two-tail	2.02	

Relationship of total flavonoid content in both locations:

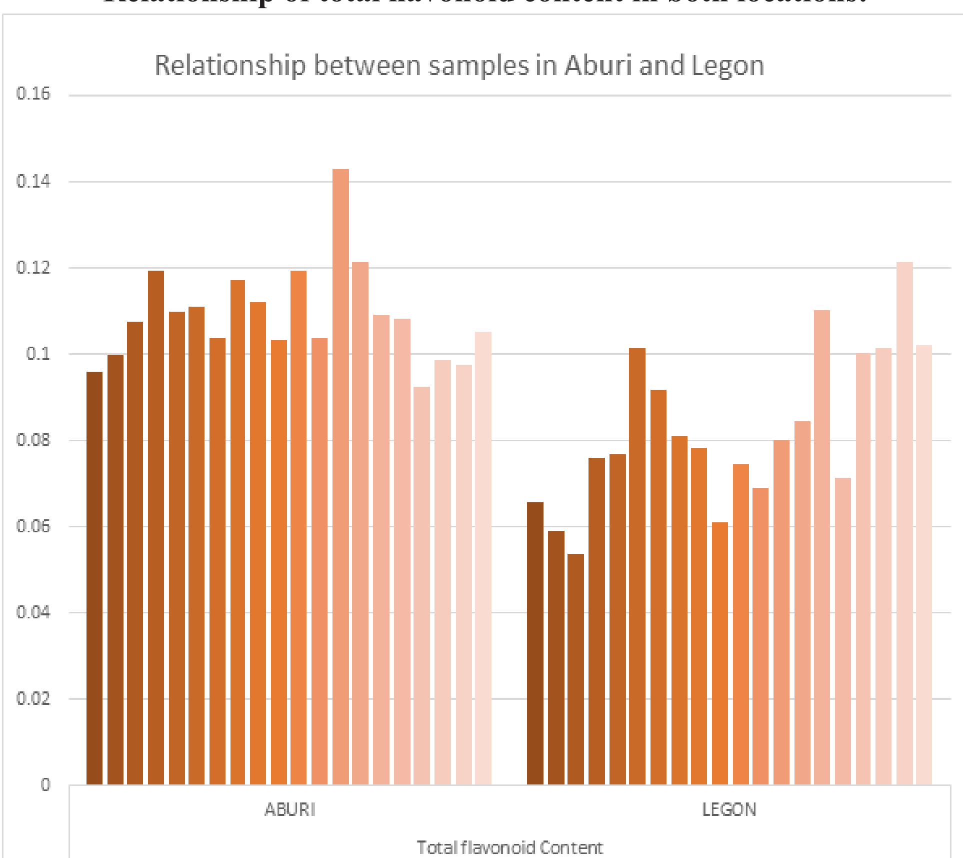

Figure 3: A bar graph showing the relationship between the total flavonoid content found in the two locations.

DISCUSSION

Patterns of variation of total phenolic content, total flavonoid content, and antioxidant activity between two populations of *Amaranthus viridis.*

This study examined the phytochemical composition of *Amaranthus viridis* from two ecological locations: Aburi Botanical Garden, a semi-deciduous forest ecosystem, and Legon Botanical Garden, a coastal savannah area. The results showed significant differences in total phenols, total flavonoids, and antioxidant activity between the two locations. The study found that Aburi Botanical Garden's semi-deciduous forest environment likely provides a more favorable environment for the accumulation of phenolic compounds, leading to increased phenol production. Flavonoids, known for their antioxidant properties, are essential for the plant's defense mechanisms.

The study also found a moderate difference in antioxidant activity between the two locations, with Aburi Botanical Garden exhibiting higher antioxidant activity compared to Legon Botanical Garden. This is attributed to the potentially elevated levels of total phenols, which contribute significantly to antioxidant capacity. The phytochemical concentration of *Amaranthus viridis* is influenced by the environmental settings of Aburi and Legon. Aburi has higher variation in total phenols, total flavonoids, and antioxidant activity due to the varied flora and environment of a semi-deciduous forest. A related study by [3], also observed that the contents of certain substances in plant species may increase under specific environmental conditions. For instance, phenolic compounds are known to be synthesized in response to environmental stress, and the semi-deciduous forest ecosystem of Aburi Botanical Garden may provide conditions that induce higher stress levels in *Amaranthus viridis*, leading to increased phenol production. Other literature also suggests that generally, the phenolic content of plants relates to the chemical composition of individual compounds, which in turn depends on a variety of factors, such as geographic variation, harvest time, and agricultural conditions [9,10]

Relationship for the three phytochemical contents investigated in both locations.

The bar graph depicting the total phenolic content in samples collected from Aburi Botanical Gardens and Legon Botanical Garden unmistakably illustrates a notable disparity. Most of the samples from Aburi consistently displayed slightly higher levels of phenolic compounds compared to those from Legon. This discrepancy could be attributed to several factors. Firstly, the variation in environmental conditions between the two locations may play a significant role. Aburi Botanical Gardens, nestled in the Akwapim ridge within the Tropical Rainforest Zone, experiences a more stable and favourable climate characterized by consistent rainfall patterns and moderate temperatures. In contrast, Legon Botanical Garden, with its Coastal Savannah ecological zone, may exhibit slightly different microclimatic conditions due to its specific geographical features. These environmental variations could influence the biosynthesis and accumulation of phenolic compounds in Amaranthus viridis, thus resulting in higher concentrations in the samples from Aburi. Additionally, differences in soil composition, nutrient availability, and other edaphic factors might also contribute to the observed variation in phenolic content [11].

Similar to the trend observed for total phenolic content, the bar graph representing total flavonoid content in samples from Aburi and Legon reveals a consistent pattern. All 20 samples from Aburi exhibited higher levels of flavonoids compared to those from Legon. This finding suggests a robust relationship between geographical location and flavonoid accumulation in Amaranthus viridis. The environmental factors discussed earlier, including climate, soil properties, and microclimatic variations, likely contribute to this observed difference. Flavonoids, known for their antioxidant and health-promoting properties, are synthesized by plants in response to various environmental stressors, including UV radiation, temperature fluctuations, and pathogen attacks [12,13,14]. Therefore, the comparatively favourable environmental conditions in Aburi Botanical Gardens may stimulate greater flavonoid biosynthesis in Amaranthus viridis plants, resulting in higher concentrations of these compounds in the samples collected from that location

Also, the bar graph illustrating the total antioxidant property of samples from Aburi and Legon reinforces the overarching trend observed for both total phenolic and flavonoid contents. Without exception, all 20 samples from Aburi demonstrated higher antioxidant activity compared to those from Legon. This finding underscores the vital role of environmental factors in modulating the antioxidant potential of Amaranthus viridis. Antioxidants, including phenolic compounds and flavonoids, play a crucial role in scavenging reactive oxygen species (ROS) and mitigating oxidative stress-induced damage in living organisms [15,16,17]. The significantly higher antioxidant activity observed in samples from Aburi suggests a potential health advantage associated with the consumption of *Amaranthus viridis* sourced from that location. However, it is essential to acknowledge the complex interplay of multiple factors influencing antioxidant activity, including phytochemical composition, bioavailability, and synergistic interactions among different compounds. Further studies elucidating the mechanistic basis of antioxidant activity in Amaranthus viridis from diverse geographical regions could facilitate the development of targeted strategies for enhancing the nutritional and therapeutic value of this indigenous leafy vegetable.

CONCLUSION

In conclusion, this study explored how different ecological settings, such as the semi-deciduous forest of Aburi Botanical Garden and the coastal savannah of Legon Botanical Garden, impacted the phytochemical composition of Amaranthus viridis. The findings highlighted that while total flavonoid concentration remained relatively stable across both zones, Aburi's forest environment fostered higher total phenolic content and subsequent antioxidant activity. These results underscored the adaptability of Amaranthus viridis to varying ecological conditions, shedding light on the dynamic relationship between nature and phytochemistry. This understanding had implications for agriculture, healthcare, and nutrition, as it offered insights into optimizing cultivation practices for higher phytochemical yields. For instance, in environments with greater environmental stressors, the plant naturally produced more phenolic compounds, enhancing its antioxidant properties. Conversely, in more favourable conditions, cultivation techniques could be adjusted to promote phenolic production, further boosting the plant's antioxidant capacity

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