



## Antinutrients Properties and Antioxidant Activity of Some Nigerian Leafy Vegetable Plants

Faith C. Ogbonnaya<sup>1\*</sup>, Gogonte H. Amah<sup>1</sup>, Babafemi T. Ogunbiyi<sup>1</sup>, Ngozi A. Ukangwa<sup>1</sup>, Oluwaseun A. Adewole<sup>1</sup>, Emmanuel O. Akani<sup>2</sup>, Lois O. Oladele<sup>1</sup>

<sup>1</sup>Department of Biochemistry, Babcock University, Ogun State, Nigeria

<sup>2</sup>Department of Biochemistry, University of Ibadan, Oyo State, Nigeria

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### ABSTRACT

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The leaves of red cocoyam (*Colocasia esculenta*), tropical nettle (*Laportea aestuans* L.), carrot (*Daucus carota* L.), cassava (*Manihot esculenta*, Crantz), and sesame (*Sesamum indicum*) are known for their numerous health benefits due to their high levels of phytochemicals like phenols and flavonoids. This study aims to evaluate the antinutrients properties and antioxidant activity of the leaves of these plants. The moisture and ash content were determined using the AOAC method. The total phenols, total flavonoids, vitamin A, total tannins, and total cyanogenic glycosides contents were determined following standard procedures. The antioxidant activity was assessed by the 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-Azino-bis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS<sup>+</sup>) radical scavenging assays. Among the plants, *S. indicum* leaves was found to have the highest total phenols and flavonoids contents with values of 825.95 mg GAE/100 g, and 7.80 mg QE/100 g, respectively. The leaves of *C. esculenta*, *D. carota*, and *M. esculenta* had the highest vitamin A (7.63 mg/100 g), total tannins (112.27 mg/100 g), and total cyanogenic glycosides (1792.65 mg/100 g) contents, respectively. The plants showed moderate to high antioxidant activity with *D. carota* exhibiting the highest DPPH radical scavenging activity (60.91%), while *M. esculenta* showed the highest ABTS radical scavenging activity (94.63%). The presence of antioxidant molecules and high radical scavenging activity suggest that these plants are potential sources of nutraceuticals, which could be harnessed in the management of oxidative stress-related diseases. However, the method of preparation that would reduce antinutrients, improve digestibility, and reduce toxicity of these plants should be adopted.

**Keywords:** Antioxidants, Antinutrients, Nutraceuticals, Phytochemicals.

### Introduction

Plants are major components of human diet due to their high nutritional and therapeutic values.<sup>1</sup> They occupy a strategic level in the food chain, making a majority of organisms to directly or indirectly depend on them.<sup>2</sup>

The synthesis of secondary metabolites (phenols and flavonoids) contributes to strengthening plants' defense mechanisms against diverse stressors, including biotic factors, such as predators, and abiotic factors, such as oxidants.<sup>3,4</sup> These metabolites, termed phytochemicals, are bioactive compounds that show myriads of activities in biological systems.<sup>5</sup>

Phytochemicals, have diverse effects on living organisms, and provide a variety of health benefits,<sup>6</sup> including preventing and curing nearly every disease known to man.<sup>7</sup> Terpenoids, carotenoids, phenolics, phytosterols, and glucosinolates are the most common bioactive substances of plant origin.<sup>8</sup> Many of these bioactive chemicals are reported to have antibacterial, anticancer, antidiabetic, antihypertensive, immunomodulatory, anti-osteoporotic, anticancer, and antioxidant activities as well as reported beneficial roles in managing cardiovascular dysfunctions.<sup>6,9,10</sup>

Importantly, phytochemicals serve as antioxidants to mop up free radicals implicated in oxidative stress that could be associated with diseases like ulcer, asthma, stroke, arthritis, Alzheimer's disease, and cancer.<sup>11</sup>

Leafy vegetables are good sources of antioxidants, they play an important role as anti-aging agents, and their cell protective mechanisms may ultimately result in low mortality when consumed copiously.<sup>12</sup> Globally, climate change has adversely affected the yield of vegetables often used in local cuisines among other impacts like infestation, outbreak of diseases and desert encroachment.<sup>13</sup> These vegetables are rich sources of nutraceuticals that can be harnessed to mitigate teeming diseases that are due to oxidative stress. Therefore, this study aim to evaluate less frequently used leafy vegetables for their antinutrients properties, as well as their antioxidant activity.

### Materials and Methods

#### Plant sample collection and authentication

*Colocasia esculenta* (red Cocoyam), *Laportea aestuans* L (Tropical nettle weed), *Daucus carota* L (Carrot leaves), *Manihot esculenta*, Crantz (Cassava leaves), and *Sesamum indicum* (Sesame leaves) were obtained from a local farm in Western Nigeria (latitude, 6° 53' 16.49"N; Longitude, 3° 43' 12.11"E) in November 2022. The samples were identified, authenticated and specimen voucher references of UIH-23141, UIH-23142, UIH-23143, UIH-23144, and UIH-23149 for *C. esculenta*, *L. aestuans*, *D. carota*, *M. esculenta*, and *S. indicum*, respectively were deposited at the Herbarium of the Department of Botany, University of Ibadan.

\*Corresponding author. E mail: [ogbonnayaf@babcock.edu.ng](mailto:ogbonnayaf@babcock.edu.ng)

Tel: +2347032365117

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### Sample preparation

The plants were sorted to remove debris and extraneous matter, rinsed in plentiful water to remove dirt and dried to a constant weight under convectional current at room temperature. Each sample was pulverized, and packed in a zip lock polyethylene bag, placed in an air-tight container, and kept in the refrigerator at  $-4^{\circ}\text{C}$  until required for analyses. Duration of sample storage was minimized, and conditions optimized to ensure sustained sample integrity.

### Determination of moisture content

The moisture content of the powdered sample was determined according to the AOAC method.<sup>14</sup> Briefly, three replicate clean, empty crucibles were dried to constant weights, in hot air oven (Surgifield Oven, SM9023A.9053A.9123A, Surgifield Medical England, England) at  $110^{\circ}\text{C}$ , and allowed to cool in a desiccator containing self-indicating desiccant and weighed ( $W_1$ ). Each ground sample (1 g) was weighed ( $W_2$ ) into the crucible and oven dried to constant Weight ( $W_3$ ). The moisture content was determined using the formulae below;

$$\% \text{ Moisture} = \left( \frac{W_2 - W_3}{W_2 - W_1} \right) \times 100 \dots\dots\dots(1)$$

### Determination of ash content

Ash content was determined according to the AOAC method.<sup>14</sup> Porcelain crucibles were dried in the oven at  $100^{\circ}\text{C}$  for 10 minutes, after cooling in a desiccator, they were weighed ( $W_1$ ). Ground sample (1 g each) was placed in the crucibles, and reweighed ( $W_2$ ). Thereafter, the samples were placed in the furnace (H-1081, Medical Instrument Cooperative, Rakoczi, Netherlands), and charred at  $550^{\circ}\text{C}$  for 4 hours. The crucibles were weighed ( $W_3$ ) after they were retrieved from the furnace. The ash content of each sample was calculated using the formulae shown in Equation 2 below.

$$\% \text{ Ash} = \left( \frac{W_3 - W_1}{W_2 - W_1} \right) \times 100 \dots\dots\dots(2)$$

### Determination of total phenol content

Methanol extract of each sample (0.1 mL of 0.1 g/mL) was mixed with 1 mL of 10% Folin Ciocalteu reagent and 0.8 mL of 1 M  $\text{Na}_2\text{CO}_3$ . After 15 min of incubation at room temperature, the absorbance of the mixture was read at 765 nm. The standard curve was prepared using gallic acid (0.01 to 0.07 g/mL in methanol).<sup>15</sup>

### Determination of total flavonoid content

Total flavonoid content of the methanol extract of the leaves of the plants was determined using colorimetric method.<sup>16</sup> A mixture containing the extract (0.1 mL), methanol (0.6 mL), 10% aluminium chloride (0.2 mL), 1 M potassium acetate (0.2 mL), and distilled water (0.1 mL) was incubated at  $25^{\circ}\text{C}$  for 30 min. The absorbance of the resulting mixture was read at 415 nm. The total flavonoid content was estimated from a calibration curve prepared using quercetin.

### Determination of Vitamin A content

Vitamin A content of each plant extract was determined spectrophotometrically according to the method described by Pearson (1970).<sup>17</sup> The powdered plant samples (0.1 g each) was mixed with petroleum ether (2 mL), and the mixture was evaporated to dryness in a water bath. The residue was rinsed with 0.2 mL of 50% chloroform acetic anhydride and 2 mL of 50% trichloroacetic acid chloroform, and thereafter the absorbance was read at 620 nm. Vitamin A content was calculated from retinol standard curve.

### Evaluation of the antinutrients properties

#### Determination of total tannin content

Total tannin content was determined according to the method described by Awe and Sodipo (2001).<sup>18</sup> Powdered plant samples (0.2 g each) was

dissolved in 20 mL of 50% methanol in a 50 mL beaker covered with foil paper, and heated in a water bath with shaker (Rex-C900, Techmel & Techmel, USA) for 1 hour. The extract was filtered using double-layered What-man No 41 filter paper into a 100 mL volumetric flask. To the extract was added 2.5 mL Folin-Ciocalteu reagent, 10 mL of 17%  $\text{Na}_2\text{CO}_3$ , and 20 mL of distilled water. The absorbance of the resulting mixture was measured at 760 nm. The average gradient factor was extrapolated from a tannin standard curve, and the percentage tannin content was calculated using the formula as shown in equation 3 below.

$$\% \text{ Tannin} = \left( \frac{A_s \times GF_A \times D_f}{W_s} \right) \times 100 \dots\dots\dots(3)$$

Where:

$A_s$  = Absorbance of sample  
 $GF_A$  = Average gradient factor  
 $D_f$  = Dilution factor  
 $W_s$  = Weight of sample

#### Determination of cyanogenic glycosides content

Cyanogenic glycosides content was determined using the alkaline picrate method as described by Onwuka and Olapade (2005).<sup>19</sup> The powdered plant sample (0.2 g each) was dissolved in 4 mL of distilled water in a corked conical flask and filtered after 12 hours. A mixture of the filtrate (1 mL), and alkaline picrate (4 mL) was incubated at  $35^{\circ}\text{C}$  for 5 minutes. The absorbance of the mixture was measured at 490 nm. The cyanogenic glycosides content was determined by extrapolation from a cyanide standard curve, and calculated using the formula as shown in equation 4.

$$\text{Cyanide } (\mu\text{g}) = \left( \frac{A_s \times GF_A \times D_f}{W_s} \right) \times 100 \dots\dots\dots(4)$$

Where:

$A_s$  = Absorbance of sample  
 $GF_A$  = Average gradient factor  
 $D_f$  = Dilution factor  
 $W_s$  = Weight of sample

#### Determination of antioxidant activity

##### DPPH free radical scavenging assay

DPPH (2,2-diphenyl-1-picryl hydrazyl) radical scavenging activity was determined using the method described by Gyamfi *et al.* (1999).<sup>20</sup> The methanol extract (0.05 mL) of each sample (0.1 g/mL) was mixed with 0.2 mL of 0.0003% DPPH. The mixture was incubated in the dark at room temperature for 35 min, after which the absorbance was measured at 520 nm using a UV-Visible spectrophotometer (SP-VG722, Houston, USA). Absorbance of DPPH solution only was recorded as the control. DPPH scavenging activity was calculated as shown in Equation 5.

$$\% I = \frac{Abc - Abs}{Abs} \times 100 \dots\dots\dots(5)$$

Where:

% I = Percentage inhibition  
 $Abc$  = Absorbance of control  
 $Abs$  = Absorbance of sample

##### ABTS radical scavenging assay

The ABTS radical scavenging assay activity was determined according to the method previously described by Re *et al.* (1999).<sup>21</sup> Briefly, a mixture of 0.2 mL aqueous extract (0.1 g/mL) and 2 mL  $\text{ABTS}^+$  solution was generated by reacting 7 mmol/L ABTS with 2.45 mmol/L potassium persulphate ( $\text{K}_2\text{S}_2\text{O}_8$ ) in aqueous solution and standardized to  $0.700 \pm 0.05$  at 734 nm. The mixture was incubated at room temperature in a dark chamber for 15 min. The absorbance of ABTS solution without the plant samples was used as the control. ABTS scavenging activity was calculated as shown in Equation 6.

$$\% I = \frac{Abs - Abs}{Abs} \times 100 \quad \dots\dots\dots (6)$$

Where:

% I = Percentage inhibition

Abs = Absorbance of control

Abs = Absorbance of sample

#### Statistical analysis

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 22.0. Data were presented as mean  $\pm$  standard deviation of triplicate measurements. Differences between means were analyzed using One-way analysis of variance at 5% level of significance. P-value < 0.05 was regarded as significant.

## Results and Discussion

### The moisture and ash contents of the plant leaves

The moisture content of the leaves studied ranged from  $4.33 \pm 0.58\%$  in *D. carota* to  $9.67 \pm 0.58\%$  in *L. aestuans* (Table 1). The moisture and ash contents of the tropical nettle weed were higher in comparison to the other leaf samples. Moisture contents of *L. aestuans* and *M. esculenta* leaves were significantly higher ( $p < 0.05$ ) than those of *S. indicum*, *D. carota*, and *C. esculenta* leaves. The ash content ranged from  $7.00 \pm 0.00\%$  in Sesame leaves to  $18.00 \pm 1.00\%$  in tropical nettle leaves. The low moisture content of the samples suggests that the plant samples are less susceptible to microbial attack, and as such would not easily deteriorate on storage.<sup>19</sup>

**Table 1:** Moisture and ash contents of the plants leaves

Plant	Moisture content (%)	Ash content (%)
<i>D. carota</i>	$4.33 \pm 0.58^c$	$14.00 \pm 0.00^b$
<i>C. esculenta</i>	$4.67 \pm 0.58^c$	$9.67 \pm 0.58^c$
<i>L. aestuans</i>	$9.67 \pm 0.58^a$	$18.00 \pm 1.00^a$
<i>M. esculenta</i>	$8.33 \pm 1.53^a$	$9.33 \pm 0.58^c$
<i>S. indicum</i>	$7.33 \pm 0.58^b$	$7.00 \pm 0.00^d$

Values are mean  $\pm$  standard deviation (SD), n = 3. Values with different superscripts letters down a column are significantly different at  $p < 0.05$ .

### Phenols and Flavonoids contents of the plants leaves

Total phenolic content ranged from  $80.79 \pm 6.51$  mg/100 g in *M. esculenta* to  $825.95 \pm 19.86$  mg/100 g in *S. indicum*. There were significant differences in the phenolic contents among the various samples, although *D. carota*, and *L. aestuans* had comparable phenolic contents (Table 2). On the other hand, the flavonoids content ranged from  $1.62 \pm 0.16$  mg/100 g in *M. esculenta* to  $7.80 \pm 0.38$  mg/100 g in *S. indicum* leaves. Among all the plants, the leaves of *S. indicum* had the highest amounts of both total phenols and total flavonoids, and these were significantly ( $p < 0.05$ ) higher than each of the other samples. The leaves of *C. esculenta* had the second highest total phenol and total flavonoid contents. It was observed that the flavonoid contents in both *S. indicum* and *C. esculenta* leaves were significantly higher than those of the leaves of the other three plants, which had comparable total phenol and flavonoid contents.

Phenols and flavonoids are bioactive secondary metabolites, which are known to possess anti-carcinogenic, anti-diabetic and cardioprotective effects through direct and indirect antioxidant effects.<sup>22-27</sup> The findings from both phenols and flavonoid contents of *S. indicum* agrees with the findings from the work of Kim *et al.* (2021)<sup>27</sup>, and Hridhya *et al.* (2023)<sup>28</sup>, who found appreciable amount of phenolic and flavonoid contents in *S. indicum* leaves ethanol extract.

### Vitamin A content of the plants leaves

Vitamin A content in the leaves of the plants ranged from  $1.14 \pm 0.06$  mg/100 g to  $7.63 \pm 0.86$  mg/100 g. *C. esculenta* leaves had the highest vitamin A contents of  $7.63 \pm 0.86$ , while the leaves of *M. esculenta* had the lowest vitamin A content of  $1.14 \pm 0.06$  mg/100 g, which was significantly ( $p < 0.05$ ) lower compared to all the other samples (Table 3).

The vitamin A content in *D. carota*, *C. esculenta*, *S. indicum*, and *L. aestuans* were higher than the recommended daily allowance (RDA) of 0.6 to 0.9 mg, and 0.6 to 0.7 mg for male and female, respectively. Vitamin A play a significant role in vision, growth, and development of individuals.<sup>29</sup> It is usually used as a supplement to prevent vitamin A deficiency especial in children between 6 to 56 months.<sup>30</sup>

### Antinutrients properties of the plants leaves

The results of the antinutrients assay are presented in Table 4. *D. carota* had the highest contents of both tannins ( $112.27 \pm 0.02$  mg/100 g), and cyanogenic glycosides ( $1792.65 \pm 0.82$  mg/100 g). *M. esculenta* had the lowest tannin content ( $19.39 \pm 0.03$  mg/100 g), while *L. aestuans* had the lowest content of cyanogenic glycosides ( $6.41 \pm 2.19$  mg/100 g).

Cyanogenic glycosides are chemical substances found in foods that, when chewed or digested, produce hydrogen cyanide, resulting in acute poisoning and a variety of chronic disorders.<sup>31</sup> Enzymatic breakdown of cyanogenic glycosides results in the production of hydrogen cyanide, which can cause acute cyanide poisoning by uncoupling electron transport chain.<sup>32</sup> Blanching has been shown to reduce cyanogenic glycoside during preparation of meal.<sup>33</sup>

Tannins, on the other hand are phenolic chemicals generated by plants as secondary metabolites.<sup>34</sup> Interactions between hydroxyl and carbonyl groups of tannins and proteins are responsible for both reversible and irreversible formation of tannin-protein complexes. This leads to inactivation of various digestive enzymes, consequently reducing protein digestibility.<sup>35,36</sup>

**Table 2:** Total phenol and flavonoid contents of the plants leaves

Plant	Total phenol content (mg/100 g)	Total flavonoid content (mg/100 g)
<i>D. carota</i>	$267.7 \pm 3.62^c$	$1.82 \pm 0.09^c$
<i>C. esculenta</i>	$390.06 \pm 22.83^b$	$6.00 \pm 0.56^b$
<i>L. aestuans</i>	$252.75 \pm 34.15^c$	$1.72 \pm 0.08^c$
<i>M. esculenta</i>	$80.79 \pm 6.51^d$	$1.62 \pm 0.16^c$
<i>S. indicum</i>	$825.95 \pm 19.86^a$	$7.80 \pm 0.38^a$

Values are mean  $\pm$  standard deviation (SD), n = 3. Values with different superscripts letters down a column are significantly different at  $p < 0.05$ .

**Table 3:** Vitamin A content of the plants leaves

Plant	Vitamin A content (mg/100 g)
<i>D. carota</i>	$6.52 \pm 0.43^a$
<i>C. esculenta</i>	$7.63 \pm 0.86^a$
<i>L. aestuans</i>	$6.13 \pm 0.02^a$
<i>M. esculenta</i>	$1.14 \pm 0.06^b$
<i>S. indicum</i>	$6.86 \pm 0.16^a$

Values are mean  $\pm$  standard deviation (SD), n = 3. Values with different superscripts letters down a column are significantly different at  $p < 0.05$ .

**Table 4:** Levels of antinutrients in the plants leaves

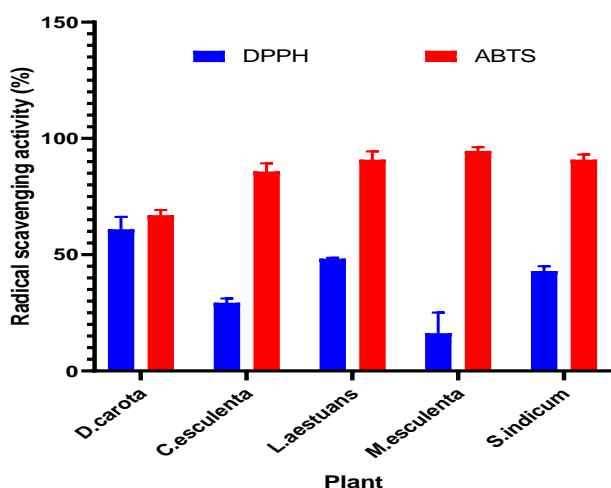
Plant	Total tannin (mg/100 g)	Total cyanogenic glycosides (mg/100 g)
<i>D. carota</i>	112.27 ± 0.02 <sup>a</sup>	110.07 ± 4.08 <sup>b</sup>
<i>C. esculenta</i>	111.80 ± 0.03 <sup>b</sup>	32.50 ± 4.02 <sup>c</sup>
<i>L. aestuans</i>	105.31 ± 0.03 <sup>d</sup>	6.42 ± 2.22 <sup>d</sup>
<i>M. esculenta</i>	19.39 ± 0.03 <sup>e</sup>	1792.65 ± 0.82 <sup>a</sup>
<i>S. indicum</i>	107.41 ± 0.04 <sup>c</sup>	23.69 ± 3.95 <sup>c</sup>

Values are mean ± standard deviation (SD), n – 3. Values with different superscripts letters down a column are significantly different at  $p < 0.05$ .

#### Antioxidant activity of the plants leaves

The results of the antioxidant assays are presented in Figure 1. The DPPH and ABTS radical scavenging assays were used as the measure of the antioxidant activity of the plants leaves. The percentage radical scavenging activity ranged from 16.23 ± 8.85% to 60.91 ± 5.33% in the DPPH assay, while in ABTS assay, the free radical scavenging activity ranged from 67.02 ± 21.22% to 94.63 ± 1.60%. *D. carota* had the highest radical scavenging activity of 60.91 ± 5.33% in the DPPH assay, while *M. esculenta* had the least. On the contrary, *M. esculenta* had the highest ABTS radical scavenging activity of 94.63 ± 1.60%, while *D. carota* exhibited the lowest level of ABTS scavenging activity.

DPPH and ABTS are both free radicals, that are used to assess the scavenging activity of samples. The DPPH assay is based on the rapid reduction of DPPH, a stable free radical, whereas the ABTS assay relies on the production of ABTS<sup>+</sup> which is gradually mopped up.<sup>37</sup> The direct and indirect antioxidant properties of a test sample are studied using ABTS and DPPH radical scavenging activity, respectively.<sup>38</sup> In the present study, *D. carota* exhibited the highest DPPH radical scavenging activity but had the lowest ABTS radical scavenging activity, while the tropical nettle weed, and sesame leaves showed the highest ABTS radical scavenging activity. Thus *D. carota*, *L. aestuans*, and *S. indicum* have excellent free radical scavenging activity compared to the other two samples (*M. esculenta* and *C. esculenta*). The presence of both direct and indirect antioxidants activities makes these plants a viable source of nutraceuticals.<sup>39</sup>



**Figure 1:** Free radical scavenging activity of the plants leaves

#### Conclusion

The leafy vegetables studied demonstrated very high antioxidant activity and could therefore, be applied to mitigate biological redox imbalance while conferring associated health benefits when consumed appropriately. The high levels of total tannin and cyanogenic glycosides in the raw vegetables suggest that the vegetables should be appropriately processed before consumption. The vegetables, which could be very good sources of minerals, should be freshly prepared for consumption as they are high in moisture. Notwithstanding, the

respective mineral nutrient compositions and the impact of processing on the nutrient and antinutrient contents of the vegetables should be studied in order to provide additional insights into how they could be best applied for optimum health benefits.

#### Conflict of Interest

The authors declare no conflict of interest.

#### Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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