

Brief Communication

Evaluation of phytochemical, proximate, antioxidant, and anti-nutrient properties of *Corchorus olitorius*, *Solanum macrocarpon* and *Amaranthus cruentus* in Ghana

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Received January 23, 2023; Accepted April 15, 2023; Epub April 15, 2023; Published April 30, 2023

Abstract: Introduction: In Ghana, *Corchorus olitorius*, *Solanum macrocarpon* and *Amaranthus cruentus* are green leafy vegetables that are customarily eaten together with a starchy staple food. The present study aimed at assessing the ethanolic leaf extract of *C. olitorius*, *S. macrocarpon* and *A. cruentus* for antioxidant capacity, phytochemical property, nutritional and anti-nutrient content. Method: Phytochemical constituent and proximate analysis were determined using standard protocols. The DPPH scavenging activity was used to determine the antioxidant activity of the ethanolic leaf extracts from the three vegetables. The antinutrients phytate and oxalate were determined by titrimetric methods of analysis. Results: Phytochemical screening revealed the presence of tannins and flavonoids in *C. olitorius*, *S. macrocarpon* and *A. cruentus*. Alkaloids and saponins were present in *C. olitorius* and *S. macrocarpon* but not in *A. cruentus*. Terpenoids, steroids, carotenoids and coumarins were absent in all the three vegetables. Proximate analysis revealed varying levels of moisture, fat, protein, ash, crude fibre and carbohydrates in the three leafy vegetables. The DPPH scavenging showed 86.71%, 71.72% and 38.86% activity for *S. macrocarpon*, *C. olitorius* and *A. cruentus* respectively. The antinutrient results revealed an oxalate level of $2.7 \pm 0.13\%$ for *C. olitorius*, $6.43 \pm 0.06\%$ for *A. cruentus* and $12.32 \pm 0.13\%$ for *S. macrocarpon*. For levels of phytates, our results revealed a $3.084 \pm 0.54\%$, $1.14 \pm 0.26\%$ and $1.71 \pm 0.27\%$ for *C. olitorius*, *A. cruentus* and *S. macrocarpon*, respectively. Conclusion: The current study has shown that *C. olitorius*, *A. cruentus* and *S. macrocarpon* possess important phytochemicals, nutrients and significant antioxidant activity, suggesting a potential of these vegetables against diverse disease, if eaten by humans.

Keywords: Antinutrient, antioxidant, phytochemicals, proximate analysis

Introduction

In sub-Saharan Africa, green leafy vegetables form a major component of most local meals because they have nutritional and/or medicinal properties [1-4]. Leafy vegetables are herbs grown for food; they can either be eaten raw or cooked. In addition to serving as important sources of several micro-minerals [3, 5], vegetables contain antioxidants which are impor-

tant for the prevention of diseases in humans [6]. For example, carrots, tomatoes, and cabbage plants have abilities to prevent several nutrient deficiency diseases in humans and animals, because they contain vitamins C and E, carotenoids, flavonoids, and thiol which act as antioxidants [7]. Antioxidants can suppress radicals and/or oxidation that cause damage to cellular components, through the formation of radical adducts which removes any free-radical

Nutritional composition of three leafy vegetables in Ghana

intermediates [1, 8]. Fortunately, plant-derived antioxidants are considered safer than synthetic types, which can be associated with toxic effects in humans and animals [9]. In this case, vegetables can also be considered a safer source of nutrients, although they may show some toxic factors (i.e., antinutrients) [10, 11].

Antinutrients are plant-derived biochemicals known for altering the nutritional contents of edible plants [10, 12]. This could pose a health risk to humans who often eat plant products and vegetables. In vegetables, some antinutrients reported in the literature include saponins, phenolics, tannins, protease inhibitors, and nitrates [13]. However, oxalates, phytates, and the latter are of health concern because they have chelating effects on several micro-nutrients [10, 14]. Phytates inhibit the absorption of minerals as well as digestion of protein and starch [14]. Oxalates can chelate dietary calcium to prevent it from being absorbed in the digestive tract [10]. Larger 'phytate-to-Fe molar ratios' than 1 prevent/or reduces the absorption of iron, although the recommended and safer ratios have been about 0.4 [11]. Calcium absorption may likely be impaired if the 'phytate-to-Ca' molar ratios in edible plants or vegetables are more than 0.17 [15]. The content of antinutrients varies among plants [12]. As such, their effects may depend on the concentration present in edible plants. Thus, the consumption of vegetables that have higher antinutrient contents will expose humans to several toxic biochemicals.

In Ghana, *Corchorus olitorius* (or jute mallow), *Solanum macrocarpon* (the African eggplant; Solanaceae) and *Amaranthus cruentus* are important green vegetables for food and medicinal purposes. The leafy parts of these vegetables are known to have a high nutritional value and medicinal properties such as antimicrobial and anti-inflammatory [12, 16]. *C. olitorius*, is a fairly branching tropical plant that belongs to the family Malvaceae. Its root and leaf are used for the treatment of heart diseases, fever, vomiting, diarrhea, and toothaches [17, 18]. Traditional medicine also employs *C. olitorius* to treat malignancies, chronic cystitis, and gonorrhoea, and as an analgesic, febrifuge, anti-inflammatory, diuretic, and cardiotoxic [19]. *S. macrocarpon* grows and can produce fruits well

throughout the year in humid subtropical and tropical zones in West and Central Africa, south-eastern Asia, south, and central Americas or the Caribbean islands [20, 21]. African eggplant (*S. macrocarpon*) bears oblong-shaped egg-like fruits used for large food. *A. cruentus* is widely common in sub-Saharan Africa where warm climatic conditions favour its growth. Its leaves are lanceolate-like, acute, and greyish-green in colour. This plant possesses five petal lobes, five stamens, and seeds with varying colours. There are numerous therapeutic uses for the leaves of *S. macrocarpon*. For instance, cooked leaves are chewed in Sierra Leone to cure throat issues, and crushed leaves are consumed in Kenya to address stomach issues [22]. *A. cruentus* have been reported to contain an active ingredient with medicinal properties such as antioxidant activity [23]. For example, gallic acid is the primary phenolic acid present in both seeds and sprouts of *A. cruentus*. The seeds of the plant also contain p-hydroxybenzoic acid, vanillic acid, p-coumaric acid, caffeic acid, cinnamic acid, Syringic acid, ferulic acid, and p-coumaric acid.

Because *C. olitorius*, *S. macrocarpon*, and *A. cruentus* leaves are widely used to prepare food and traditional herbal medicines in Ghana, a study is needed to assess their nutrient contents. Therefore, this work aimed to determine and compare the phytochemical, proximate analysis, antioxidant capacity, and antinutrient of leaves of *C. olitorius*, *S. macrocarpon*, and *A. cruentus*.

Materials and methods

Plant samples

Plant materials (*C. olitorius*, *S. macrocarpon*, and *A. cruentus*) were obtained from the Crop Research Institute of the Council for Scientific and Industrial Research (CSIR) at Kwadaso (latitude 6°41'25.7"N, longitude 1°38'57.67" W) on 18-24 April 2019. The plants were identified and authenticated by the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology, Ghana. Voucher specimens coded KNUST/HM1/L/2021/026, KNUST/HM1/L/2021/027 and KNUST/HM1/L/2021/027 for *C. olitorius*, *S. macrocarpon*, and *A. cruentus*, respectively,

Nutritional composition of three leafy vegetables in Ghana

and were deposited in the herbarium unit of the University for reference. Samples from each of these vegetables were taken for the study.

Plant sample preparation for the study

About 3 kg of each of the three green leafy vegetables was taken and then washed under running tap water followed by rinsing it with distilled water. The clean leaves were stored in a refrigerator at 4°C until needed for experimental use.

Sources of chemicals and reagents

All chemicals were purchased from Sigma Aldrich Co. Ltd., Irvine, U.K. The organic solvents were of analytical grade and purchased from BDH Laboratory Supplies (England).

Crude extracts from leafy vegetables

About 200 g of the air-dried plant materials (i.e., leaves) were subjected to a 24-hour extraction in absolute ethanol, using a Soxhlet apparatus. The extract was filtered, concentrated and evaporated to dryness using the rotary evaporator (Buchi Rota Vapor R-114) to obtain the crude extract. Three replicates for each plant species were made.

Phytochemical screening and proximate analysis

The crude extracts obtained from the three leafy green vegetables were screened for the presence of phytoconstituents using the methods described by Trease and Evans [24]. Moisture, protein, ash, crude fat and crude fiber contents were determined according to the protocol of Association of Official Agricultural Chemists, AOAC [25]. Carbohydrate and protein contents were determined according to the method used by Obeng *et al.* [26]. Determination of carbohydrate was achieved by using equation 1. Titrimetric method of analysis was used for protein determination. About 2 g of the plant sample was measured into a digestion tube having a half-filled selenium-based catalyst. About 25 ml of concentrated H₂SO₄ was then added to the plant sample. The digestion tube was placed on a digestion burner and heated slowly until the boiling ceased and the resulting solutions became clear. 10 ml of the digested sample solution was pipetted into

a decomposition flask of the Kjeldahl unit, and excess 40% NaOH apportioned into it. The ammonia produced was distilled into the conical flask. The distillate was titrated against with 0.1 N HCL solution. Percentage crude protein was calculated using equation 2. To calculate protein, total nitrogen was converted using a conversion factor (F) of 6.25. The screening and the analysis were replicated three times.

$$\text{Carbohydrate (\%)} = 100 - (\% \text{ moisture} + \% \text{ protein} + \% \text{ ash} + \% \text{ fibre} + \% \text{ fat}) \quad \text{equation 1}$$

$$\text{Crude protein (\%)} = \% \text{ Kjeldahl N} \times F \quad \text{equation 2}$$

Antioxidant (radical scavenging activity)

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging ability of the ethanolic plant extracts and standard ascorbic acid solution were determined using a modified method (Famuwagun *et al.* [20]). About 5 g of the plant extract was weighed into a centrifuge tube followed by diluting it with 50 ml of 70% ethanol. The resultant solution (or extract + 70% ethanol) was then filtered through a Whatman filter paper. About 1 ml of the extract was taken into a test tube followed by the addition of 1 ml of 0.3 mM DPPH solution. After that, the resultant solution was kept at room temperature (25-27°C) for 30 minutes. The sample was then analyzed with a UV/VIS (Mettler Toledo spectrometer) and the absorbance was 517 nm. The DPPH radical scavenging (%) was calculated using equation 3.

$$\% \text{ DPPH Scavenging} = \frac{(A_0 - A)}{A_0} * 100\% \quad \text{equation 3}$$

Where A₀ = absorbance of the control and A = absorbance of the test sample.

Determination of phytate and oxalate

Phytate and Oxalate were determined using titrimetric methods of analysis [22, 27].

Statistical analysis

Statistical Package for Social Sciences (SPSS version 25; IBM Corp., Armonk, N.Y., USA) was used for all data analyses. Data were subjected to a one-way analysis of variance to examine the assumption for significant variation between variable factors. Having observed that

Nutritional composition of three leafy vegetables in Ghana

Table 1. Phytochemical constituents present in the leaves of *C. olitorius*, *S. macrocarpon* and *A. cruentus*

Phytochemical constituents	<i>C. olitorius</i>	<i>S. macrocarpon</i>	<i>A. cruentus</i>
Saponins	+	+	-
Tannins	+	+	+
Flavonoids	+	+	+
Alkaloids	+	+	-
Terpenoids	-	-	-
Steroids	-	-	-
Carotenoids	-	-	-
Coumarins	-	-	-

(+) indicates presences of phytochemical constituent whiles (-) indicates absence.

factors significantly caused variation in the data, the difference between 'mean \pm standard error (SE)' of the variable was determined, using a post-hoc analysis (Least Significant Difference (LSD) at $P < 0.05$).

Results

Phytochemical constituents

Results for the phytochemical composition of *C. olitorius*, *A. cruentus*, and *S. macrocarpon* are shown in **Table 1**.

Proximate analysis

The three leafy vegetables *C. olitorius*, *S. macrocarpon*, and *A. cruentus* were tested for their moisture, fat, protein, ash, crude fibre and carbohydrate contents. From our results (**Table 2**), *A. cruentus* recorded the highest moisture value with $17.4071 \pm 0.11\%$ followed by *C. olitorius* which had a moisture content of $14.2184 \pm 0.09\%$. *Solanum macrocarpon* had the lowest moisture content of $13.6162 \pm 0.16\%$. Results for the high moisture content suggest that *A. cruentus* will have a shorter shelf life compared to the other leafy vegetables. Results of fat content showed that *A. cruentus* had the highest amount of fat ($9.3926 \pm 0.74\%$). *Solanum macrocarpon* had a fat content of $8.9382 \pm 0.90\%$, whiles *C. olitorius* had the lowest fat content of $8.6562 \pm 0.68\%$. Ash is the inorganic residue remaining after the water and organic matter have been removed by burning. From our results, the ash content was high in *A. cruentus* ($2.5895 \pm 0.02\%$) as compared with *S. macrocarpon* and *C. olitorius* which had ash contents of $1.6402 \pm 0.02\%$ and $1.2592 \pm 0.01\%$, respectively.

Antioxidant

The DPPH antioxidant activities of the three leafy vegetables are shown in **Figure 1**. The results showed that *S. macrocarpon* had the highest antioxidant activity of 86.71% followed by *C. olitorius* having 71.72% and *A. cruentus* having the lowest value of 38.86%. There were significant differences ($P < 0.05$) in the DPPH scavenging abilities of the three leafy vegetables.

Antinutrients

Table 3 shows the levels of oxalate and phytate in the three green leafy vegetables. *C. olitorius* had the highest amount of phytate, whereas *A. cruentus* had the lowest amount. In the case of oxalate, *S. macrocarpon* had the highest amount whereas *C. olitorius* had the lowest.

Discussion

Phytochemical composition determines the pharmacological and biochemical activity of a plant [28]. After the selected three leafy vegetables were tested for their phytochemical constituents, the results (**Table 1**) revealed that saponins, tannins, flavonoids, and alkaloids are present in *C. olitorius* and *S. macrocarpon*. We did not detect the presence of terpenoids, steroids, carotenoids, and coumarins in *C. olitorius* and *S. macrocarpon*. For *A. cruentus*, tannins and flavonoids were present but not alkaloids, terpenoids, steroids, carotenoids, saponins, and coumarins. Tannins can protect the body tissues against the action of free radicals which cause cellular aging processes [29]. Flavonoids help in the prevention of cancer, heart disease, asthma, and stroke, while saponins have been shown to possess anti-inflammatory activity [30]. Phytochemicals such as terpenoids, alkaloids, and steroids have pharmacological activities which can be wound-healing, antimicrobial, antioxidant, or antiplasmodial [31]. Based on the phytochemicals observed in these vegetables, it suggests that consumption of these leafy vegetables may result in preventing the body from several diseases.

Proximate analysis gives information about the nutritional value of food samples. Food that is

Nutritional composition of three leafy vegetables in Ghana

Table 2. Results for moisture, fat, protein, ash, crude fibre and carbohydrate content present in the three leafy vegetables *C. olitorius*, *A. cruentus* and *S. macrocarpon*

Plant	% Moisture	% Fat	% Protein	% Ash	% Crude fibre	% Carbohydrate
<i>C. olitorius</i>	14.2184 ± 0.09	8.6562 ± 0.68	0.0207 ± 0.01	1.2592 ± 0.01	33.6147 ± 0.58	0.03
<i>A. cruentus</i>	17.4071 ± 0.11	9.3926 ± 0.74	0.0203 ± 0.02	2.5895 ± 0.02	42.1365 ± 0.29	28.48 ± 0.01
<i>S. macrocarpon</i>	13.6162 ± 0.16	8.9382 ± 0.90	0.0128 ± 0.01	1.6402 ± 0.02	44.1457 ± 0.47	31.68 ± 0.04

Data are presented as mean ± standard deviation.

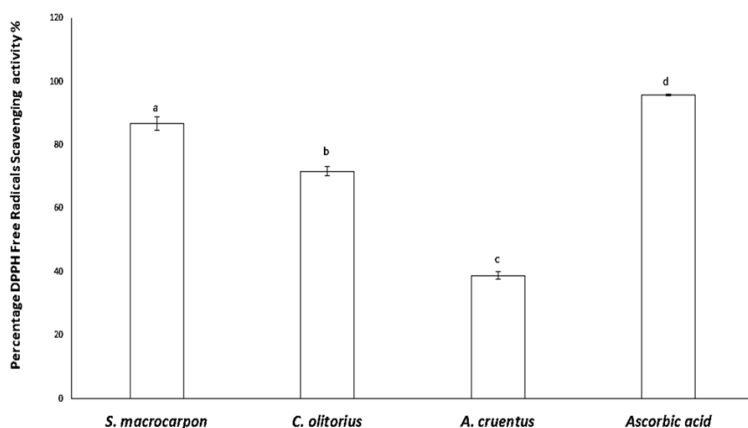


Figure 1. DPPH radical scavenging activity of three vegetables. Letters indicate significant difference (LSD; $P < 0.05$). Different letters (a, b, c, d) on the bar indicates significant difference in the scavenging activity.

Table 3. Results for phytate (%) and oxalate content (mg/100 g) present in *C. olitorius*, *A. cruentus* and *S. macrocarpon*

Plant	Phytate (mean ± SD)	MPL for phytate	Oxalate (mean ± SD)	MPL for oxalate
<i>C. olitorius</i>	3.0840 ± 0.54 ^a	200	2.7912 ± 0.13 ^c	50
<i>A. cruentus</i>	1.1402 ± 0.26 ^c	200	6.4379 ± 0.06 ^b	50
<i>S. macrocarpon</i>	1.7151 ± 0.27 ^b	200	12.3355 ± 0.13 ^a	50

Data are presented as Mean ± standard deviation. Different letters (a, b, c) against values suggest a significant difference (LSD, $P < 0.05$). MPL represent maximum permissible limits for human consumption [27].

rich in fibre has counteractive action against colon cancer and also helps in the treatment of illness [32]. For percent crude fibre, *A. cruentus* recorded a percentage of $42.1365 \pm 0.29\%$, *S. macrocarpon* had $44.1457 \pm 0.47\%$ and *C. olitorius* yielded a percentage of $33.6147 \pm 0.58\%$. Protein contents for *C. olitorius*, *A. cruentus* and *S. macrocarpon* were $0.0207 \pm 0.01\%$, $0.0203 \pm 0.02\%$, and $0.0128 \pm 0.01\%$, respectively. Carbohydrate contents for *C. olitorius*, *A. cruentus* and *S. macrocarpon* were 42.256%, 28.48%, and 31.68%, respectively. A previous study by Samuel et al. [33] reported that *C. olitorius* vegetable is composed of moisture (84.95%), crude protein (1.58%), crude fat

(0.20%), crude fibre (2.03%), ash (2.45%) and carbohydrate (10.82%). A study by Oluwole et al. [34] reported that *A. cruentus* cultivated in Nigeria had a moisture content ($16.11 \pm 1.35\%$), protein ($10.09 \pm 0.72\%$), crude ($10.68 \pm 1.13\%$), fat ($1.62 \pm 1.39\%$) and ash ($30.88 \pm 1.27\%$). Moisture, crude fibre and fat contents in the green leafy part of *A. cruentus* obtained in this study were comparably higher than those in a recent report [31]. According to Oyebamiji and Ayeni [35], *S. macrocarpon* largely grown in Nigeria has moisture, crude protein, fat, carbohydrate, ash and fibre contents of 87, 2.1, 1.11, 4.4, 2.06 and 2.89%, respectively. In this study, we observed that the fat, crude fibre, and carbohydrate contents of *S. macrocarpon* were high, although its moisture, protein and ash contents were low.

While free radicals are a natural phenomenon that occurs in cells, an excessive amount can have a negative effect [36]. Antioxidants are phytochemicals that protect cells against damage caused by the activity of free radicals [9]. The antioxidant abilities of plants to remove free radicals that may be found in animals and humans are dependent on their constituents [28]. In this study the antioxidant activity from ethanolic extract of three leafy vegetables was examined, using the DPPH (1,1 diphenyl-2-picryl Hydrazyl) radical scavenging method. Among the three vegetables studied, *S. macrocarpon* showed the most potent DPPH scavenging activity. Comparatively, the DPPH scavenging activity of ethanolic extract of *S. macrocarpon* and *A.*

Nutritional composition of three leafy vegetables in Ghana

Table 4. Reported anti-nutrient composition of some green leafy vegetables

Plant	Identified antinutrients and their contents	Reference
<i>Solanum spp.</i>	Oxalate (129.59-311.32 mg/100 g) Tannin (100.98-370.14 mg/100 g) Flavonoid (41.78-47.35 mg/100 g) Phytate (41.78-143.30 mg/100 g)	[42]
<i>Amaranthus viridis</i>	Oxalate (5.52 mg/100 g) Phytate (2.02 mg/100 g)	[43]
<i>Alphanostylis leptantha</i>	Oxalate (5.87 mg/100 g) Phytate (2.05 mg/100 g)	[43]
<i>Portulaca deracea</i>	Oxalate (96.00 mg/100 g) Tannin (465.00 mg/100 g)	[44]
<i>Telfeira occidentalis</i>	Hydrocyanic acid (31.0 mg/100 g) Oxalate (570 mg/100 g) Phytic acid (7.50 mg/100 g)	[44]

Conclusion and future work

Findings from this study revealed that the three green leafy vegetables contain essential nutrients, phytochemicals and antioxidants which will be beneficial to human health. Also, the study showed that the levels of phytate, and oxalate found in the three vegetables were within the permissible limits for human consumption. In this current

study, we examined the levels of oxalate and phytate, future studies will focus on the levels of other important antinutrients such as tannins present in the plants. Also, other antioxidant bioassays such as ferric-reducing ability potential assay and ABTS acid scavenging activity will be examined. Furthermore, future work will determine the total flavonoid content (TFC) and total phenol content (TPC) for the three plants considered in this work.

cruentus in our work was higher than that reported by Obeng *et al.* [26]. This observation may be due to the influence of geographical location on plants and their variations on constituents. Also, the solvent used for the extraction may account for the differences between our observation and that of [26]. In a previous study to access the antioxidant potential of the ethanolic extract of *S. macrocarpon*, Eletta *et al.* [37] reported a percentage inhibition of 75.61%. Previous studies have shown a positive correlation between alkaloids and DPPH scavenging ability [38, 39].

We thank Mr. Clifford Osafo Asare of the Department of Herbal Medicine, Faculty of Pharmacy and Pharmaceutical Sciences, Kwame Nkrumah University of Science and Technology for his assistance in identifying and authenticating the botanical names of the plants used in this study. The authors also wish to thank the Department of Food Science and The Department of Chemistry, all of KNUST.

In this study, we assessed the levels of oxalate and phytate in the leaf of *C. olitorius*, *A. cruentus*, and *S. macrocarpon* which are common green leafy vegetables in Ghana. High levels of antinutrients in food are known to reduce the absorption and utilization of food in human systems [27, 40]. For example, phytate and oxalate are capable of forming chelates around Ca and Mg, thereby, causing poor absorption from the intestine [41]. In this current study, the estimated levels of oxalate and phytate of the three vegetables were within the permissible limits for human consumption [27]. However, heat treatments during cooking and boiling have been observed for reducing antinutrient contents in vegetables [42]. Therefore, oxalate and phytate contents in *C. olitorius*, *A. cruentus*, and *S. macrocarpon* could be further reduced through proper cooking. In **Table 4**, we present the levels of oxalate and phytate contents of some green leafy vegetables in previous reports.

Acknowledgements

None.

Disclosure of conflict of interest

None.

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Nutritional composition of three leafy vegetables in Ghana

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