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Tropical Leafy Vegetables as Valuable Sources of Carotenoids and Phenolics

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Authors' contributions

This work was carried out in collaboration between all authors. Author LZ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PO and AZ managed the analyses of the study. Author SN managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Leafy vegetables play an important role as essential components of diet for rural populations in tropical Africa. This study was undertaken to provide a more comprehensive characterization of carotenoids and phenolics in these neglected plant foods. For this, 12 leafy vegetables widely consumed in Côte d'Ivoire were selected, washed and oven-dried (50°C/3 days) before High-Performance Liquid Chromatography (HPLC) analysis of carotenoids and phenolics compounds. Carotenoids contents were significantly different ($p < 0.05$) with lutein (43.68 ± 4.89 – 513.91 ± 5.68 µg/g dw) and all trans-β-carotene (22.62 ± 1.54 – 222.61 ± 5.63 µg/g dw) as major constituents. The calculated retinol activity equivalent (RAE) of β-carotene-rich leafy vegetables in this study ranged between 1.54 and 2.52 mg/100 g. The values of total phenolics ranged from 179.66 ± 11.33 mg/100 g dw in *Corchorus olitorius* to 436.48 ± 1.73 mg/100g dw in *Abelmoschus esculentus.* Three (3) flavonoids: quercetin (0.79 – 8.36 µg/g dw), catechin (0.39 – 5.65 µg/g dw) and kaempferol (0.76 – 29.11 µg/g dw) were quantified in the selected leafy vegetables. Chlorogenic

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acid (0.94 – 17.01 µg/g dw) was the most quantified phenolic acid in the leaves. Antioxidant activity evaluation of the leaves showed that 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) radical scavenging activity ranged between 19.63 and 65% with *Solanum melongena* showing the highest value (65%). For 2,2'-Azino-Bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS scavenging activity, *Myrianthus arboreus* recorded the highest value (76.66%) compared to other leaves. All these results suggest that the studied leafy vegetables are potential sources of carotenoids and phenolics and their consumption in sufficient amount may contribute to human health improvement.

Keywords: Leafy vegetables; carotenoids; phenolics; antioxidant activity.

1. INTRODUCTION

Plant-based foods such as fruits, vegetables, whole grains, nuts and legumes are rich in phytochemicals defined as bioactive non-nutrient having beneficial health effects [1]. Indeed, these compounds may help to reduce the risk of chronic diseases such as cancer, coronary heart disease, stroke, arthritis, diabetes and inflammatory bowel diseases [1,2]. Dietary phytochemicals can be classified into various groups as phenolics, nitrogen-containing compounds, alkaloids, organosulfur compounds, phytosterols and carotenoids. Among these groups, phenolics and carotenoids are the most studied related to human health and well-being [1,3].

Phenolics are composed of a wide range of heterogenic compounds that have at least one aromatic ring possessing more than one hydroxyl groups. They are products of secondary metabolism in plants and can be grouped into flavonoids and non-flavonoids (phenolic acids, stilbenes, coumarins, lignans and tannins) [4]. Phenolic compounds have numerous bioactive effects such as antioxidant, anti-inflammation, detoxification, immune protection, hormone modulation, anti-tumour, cardioprotection, anti-diabetes, neuro-protection, anti-allergy and endothelial protection [5,6]. The phenol explorer database indicated spices and dried herbs, cocoa products, coloured berries, flaxseed, nuts and some vegetables as richest sources of phenolic compounds [7].

As for carotenoids, they are fat soluble pigments derived from a 40- carbon basal structure of isoprene units and categorized as carotenes (β-carotene, α-carotene and lycopene) and xanthophylls (zeaxanthin, lutein, α and β-cryptoxanthin) [8,9]. Carotenoids can also be divided into two groups; those that are a precursor of vitamin A (β-carotene, α-carotene and β-cryptoxanthin) and the second group composed of carotenoids without vitamin A activity (zeaxanthin, lutein and lycopene) [10]. Apart from vitamin A activity that is important for growth, reproduction and immune system, other important biologic functions are attributed to carotenoids. Indeed, carotenoids can act as antioxidants through deactivation of free radicals and prevent therefore some pathologic disorders such as cancer, cardiovascular disease, and macular degeneration [11,12]. The major food sources of carotenoids are pigmented fruits, juices and vegetables with yellow-orange vegetables and fruits providing most of the β-carotene and α-carotene [13].

With regard to the health benefits of bioactive components such as carotenoids and phenolics, there is a growing awareness of promoting fruit and vegetable consumption in both developed and developing countries [14]. In developing countries from tropical Africa, traditional leafy vegetables have long been important components in diets as they are ingredients of soups or sauces that accompany carbohydrate staples. Indeed, reports on the diversity of traditional leafy vegetables in sub-Saharan Africa indicate more than 20 species of nutritional importance and used in daily diets [15]. In addition, leafy vegetables are low-cost quality nutrition foods for population and they provide important sources of employment in peri-urban areas because of their short production systems and relatively high yield [16].

Despite the abundance of traditional leafy vegetables, they still remain underexploited and underutilized due to increasing urbanization (preference for exotic vegetables rather than traditional ones) and inadequate scientific information on their nutritional profile especially bioactive components. The aim of this study was to provide a more comprehensive characterization of carotenoids and phenolics, in neglected traditional leafy vegetables from tropical Africa and especially those consumed in Côte d'Ivoire (West Africa). This study is significant in that it could greatly contribute to

increasing the consumption, cultivation and commercialization of these vegetables.

2. MATERIALS AND METHODS

2.1 Chemicals

The solvent (methanol, dichloroethane, methyltertiary-butyl diethyl ether and formic acid) were all HPLC grade and purchased from Fisher Scientific. The purified carotenoids standards (lutein, all-trans-β-carotene, 13-cis-β-carotene, 9-cis-β-carotene, α-carotene and β-apo-8-carotenal) were from Sigma-Aldrich while those of phenolics (gallic acid, caffeic acid, chlorogenic acid, quercetin, catechin and kaempferol) were from ACROS Organics and Sharlau. The reagent (Folin-ciocalteu, BHT, DPPH and ABTS) were analytical grade and purchased from Merck.

2.2 Leafy Vegetables

Twelve (12) leafy vegetables widely consumed in Côte d'Ivoire were selected (Table 1). All the leaves were collected at maturity from a peri-urban farmland (latitude: 5°19′14″ North; Longitude: 4°22′59″West) located in Abidjan District (Côte d'Ivoire). The collected leaves were authenticated by National Floristic Center (University Felix Houphouët-Boigny, Abidjan), washed several times with distilled water and drained at ambient temperature. Then, the leaves were oven-dried (50°C/3 days) and ground into powder with a laboratory crusher. All the dried and powdered samples were stored at -18°C in an airtight container before further experimentation.

2.3 Carotenoids Analysis

2.3.1 Carotenoids extraction

Total carotenoids samples were extracted using hexane after saponification method [17]. For this, powdered samples (ca 50 mg) were mixed with 5 mL of ethanol containing BHT (0.1%, w/v) and the whole mixture was heat in a water bath at 85°C for 5 min. Then, 400 µL KOH (80% in water) was added for saponification and the suspension was mixed using vortex for 20 s and heat in a water bath at 85°C for 5 min. The tubes containing the reaction mixture was placed in ice after introducing 3 mL deionised and total carotenoids was extracted three times with 4 mL of hexane. β-apo-8-carotenal was used as internal standard and was added after saponification. The combined extracts were dried under nitrogen and reconstituted in 1 mL (v/v) methanol-dichloroethane mixture.

2.3.2 HPLC analysis

For the carotenoids identification and quantification 25 µL of extract was injected into the 717 autosampler HPLC system (Waters, USA) consisting of a C_{30} YMC carotenoid column (4.6 x 250 mm, 3 µm), 1525 binary HPLC pump, and a 996 photo-diode array (PDA) detector. The HPLC solvent gradient included methanol-water (92:8, v/v) with 10 mM ammonium acetate (solvent A) and 100% methyl tertiary-butyl diethyl ether (solvent B). Samples were analyzed at 1 mL/min with a 30 min linear gradient from 70 to 40% of solvent A. Lutein, β-carotene (including all- trans, 13-cis and 9- cis) and α-carotene were identified and quantified using HPLC-purified standards. Chromatograms were generated at 450 nm.

2.4 Retinol Activity Equivalent (RAE)

The calculation of retinol activity equivalent (RAE) of leafy vegetables was based on the bioconversion factor defined by the Institute of Medicine (IOM) as 12 µg β-carotene to 1 µg of retinol (12:1) and α-carotene and β-cryptoxanthin as 24:1 [18].

2.5 Phenolics Analysis

2.5.1 Total phenolics determination

Total phenolics were extracted according to Hertog et al. [19] with modification as follow: approximately 50 mg of grounded plant material was mixed with 4 mL of methanol (80%, v/v) and sonicate for 5 min. After sonication, 1 mL of 6 M HCl was added and the mixture was heated in water bath at 50°C for 30 min. Afterwards, the whole mixture was cooled in ice and subjected to centrifugation (6700g; 10 min). Then, the supernatant was collected for further analysis. The quantification of phenolics was performed by using Folin-Ciocalteu's method [20]. 200 µL of phenolics extract was mixed with 800 µL of deionized water in a test tube. The mixture was oxidized with 1 mL of Folin–Ciocalteu's reagent and neutralized by 1 mL of 2% (w/v) sodium carbonate. Then 4 mL of deionized water was added and the reaction mixture was incubated for 30 min before measuring absorbance at 745 nm (Genesys UV-Vis spectrophotometer, USA). The total phenolics content was obtained using a calibration curve of gallic acid (100 µg/mL) as standard.

Table 1. Common and local names of selected tropical leafy vegetables

Leafy vegetables	Common name	Local name
Abelmoschus esculentus	Okra	Gombo
Amaranthus hybridus	Green amaranth	Boronbrou
Basella alba	Indian spinach	Epinard
Celosia argentea	Common cockscomb	Soko
Colocasia esculenta	Taro	Taro
Corchorus olitorius	Jew's mallow	Kplala
Hibiscus sabdariffa	Roselle	Dah
Ipomea batatas	Sweet potato	Patate
Manihot esculenta	Cassava	Manioc
Myrianthus arboreus	Bush pineapple	Tikliti
Solanum melongena	Eggplant	Aubergine
Talinum triangulare	Ceylon spinach	Mamichou

2.5.2 HPLC analysis

The phenolics extract was filtered through a 0,45 µm Corning syringe filter prior to 20 µL injection into the 717 autosampler HPLC system (Waters, USA) equipped with a 1525 binary pump, and a 996 photo-diode array (PDA) detector. The column used for phenolics separation was a C_{18} Sunfire (4.6 x 250 mm, 5 µm). The HPLC solvent gradient included water/formic acid (99.9:0.1, v/v) (solvent A) and 100% methanol (solvent B). Samples were analyzed at 1 mL/min with a 65 min linear gradient from 95 to 50% of solvent A. Gallic acid, caffeic acid, chlorogenic acid, quercetin, catechin and kaempferol were identified and quantified using HPLC-purified standards. Chromatograms were generated at 280 nm.

2.6 Antioxidant Activity

The DPPH test method [21] was used to assess the DPPH free radical scavenging potential of phenolics extract. Thus, 500 µL of phenolics extract was mixed with 1.5 mL DPPH 0.135 mM. The mixture was incubated in the dark at room temperature for 90 min and absorbance was read at 517 nm (Genesys UV-Vis spectrophotometer, USA) against control (DPPH solution).

For the ABTS test the method described by Re et al. [22] was performed. For this, 500 µL of phenolics extract was mixed with 1.5 mL ABTS fresh solution (OD = 0.7 – 1). The mixture was incubated in the dark at room temperature for 6 min and absorbance was read at 734 nm (Genesys UV-Vis spectrophotometer, USA) against control (ABTS solution).

The percentage of scavenging activity (DPPH or ABTS) was calculated as follows:

$$Scavenging\ activity = \frac{(Ac - As) \times 100}{Ac}$$

Ac: Absorbance of control
As: Absorbance of sample

2.7 Statistical Analysis

All analyses were carried out in triplicates and data expressed as means ± standard deviation. One way analysis of variance (ANOVA) and Duncan's multiple range test (DMRT) were carried out to assess significant differences between means ($p<0.05$) using XLStat 2017 software.

3. RESULTS AND DISCUSSION

3.1 Carotenoids Profile and Retinol Activity Equivalent

The chromatographic profile of carotenoids is shown by the Fig. 1. The identified carotenoids were lutein, α-carotene, 13-cis-β-carotene, all trans-β-carotene and 9-cis-β-carotene.

Carotenoids contents were significantly different ($p < 0.05$) from one leafy vegetable to another (Table 2). Lutein (43.68 ± 4.89 – 513.91 ± 5.68 µg/g dw) and all trans-β-carotene (22.62 ± 1.54 – 222.61 ± 5.63 µg/g dw) were the major carotenoids while α-carotene, 13-cis-β-carotene and 9-cis-β-carotene were the minor carotenoids representing less than 20% of total identified carotenoids. These results corrobate the findings that carotenoids pattern of leaves are mainly

based on lutein (about 45%) and β-carotene (about 25-30%) as reported by Rodriguez-Amaya [23]. Considering lutein contents, *A. esculentus* leaves recorded the highest value (513.91 ± 5.68 µg/g dw) followed by *I. batatas* (422.60 ± 1.01 µg/g dw), *C. esculenta* (383.92 ± 8.93 µg/g dw) and *M. esculenta* (376.79 ± 34.90 µg/g dw). These leafy vegetables may be considered as good sources of lutein compared to spinach (59.3 – 79 µg/g) and kale (48 – 114.7 µg/g) which are known as high sources of lutein on fresh weight basis [24]. Thus, they could play an important role in the reduction of cataract and macular degeneration because lutein is stored in the retina of human body for the maintenance of normal visual function [25]. For total β-carotene content, the values ranged from 26.61 ± 1.98 in *H. sabdariffa* to 300.86 ± 13.96 µg/g dw in *A. esculentus* leaves. Additionally to *S. melongena* (215.64 ±1.86 µg β-carotene /g), leafy vegetables with high content in lutein (*A. esculentus*, *I. batatas*, *C. esculenta*, *M. esculenta*) were also good sources of β-carotene. Because of their high concentrations in total β-carotene, the leafy vegetables mentioned above could potentially help alleviate vitamin A deficiency (VAD) which mainly affects children under 5 years of age in developing countries [26]. Indeed, the calculated RAE of β-carotene rich leafy vegetables in this study ranged between 1.54 and 2.52 mg/100g. Considering 50 g portion size of dried leafy vegetables, these RAE values could cover 2 to 3 fold of the Recommended Dietary Allowance (RDA) for vitamin A of children under 5 years estimated on average to 400 µg [18].

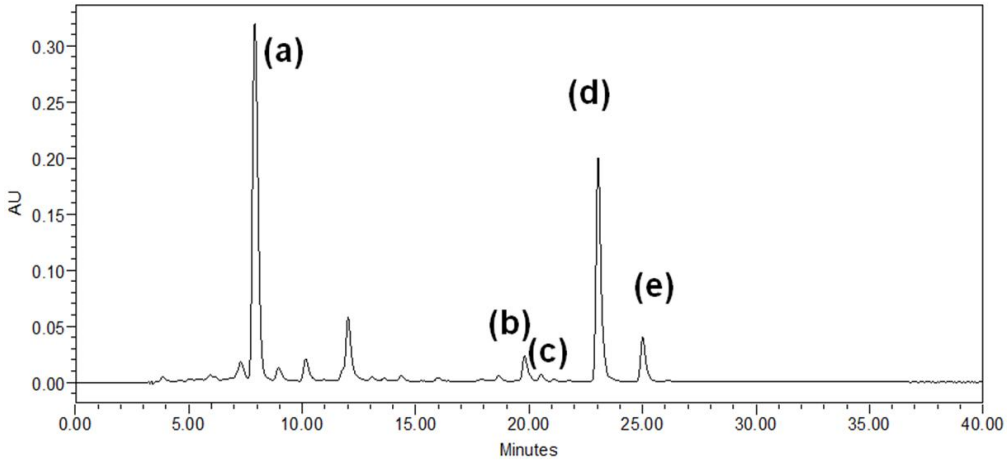

Fig. 1. Chromatrogram of carotenoids from selected tropical leafy vegetables
(a): lutein; (b): 13-cis-β-carotene; (c): α-carotene; (d): all-trans-β-carotene; and (e): 9-cis-β-carotene

Table 2. Carotenoids composition (µg/g dw) of selected tropical leafy vegetables

Leafy vegetables	Lutein	α-carotene	13-cis-β-carotene	trans-β-carotene	9-cis-β-carotene	Total β-carotene
A. esculentus	513.91±5.68^{a}	4.99±0.42^{d}	21.86±1.81^{a}	222.61±5.63^{a}	56.39±6.53^{a}	300.86±13.96^{a}
A. hybridus	248.89±1.36^{f}	0.62±0.01^{g}	8.50±1.38^{d}	100.97±0.75^{f}	19.32±0.28^{d}	128.79±2.40^{h}
B. alba	217.91±12.21^{g}	2.42±0.08^{f}	5.15±0.80^{e}	62.35±3.56^{g}	13.15±0.16^{e}	80.65±2.92^{i}
C. argentea	315.20±21.95^{e}	0.65±0.09^{g}	10.39±0.28^{cd}	116.79±4.42^{e}	20.74±0.76^{d}	147.92±5.46^{g}
C. esculenta	383.92±8.93^{c}	9.00±0.25^{c}	9.97±0.69^{cd}	144.33±2.47^{d}	26.48±0.18^{c}	180.78±2.98^{e}
C. olitorius	328.30±28.94^{de}	3.39±0.03^{e}	16.08±1.04^{b}	151.89±12.22^{d}	29.21±2.93^{c}	197.17±16.18^{d}
H. sabdariffa	43.68±4.89^{h}	nd	0.58±0.04^{f}	22.62±1.45^{i}	3.41±0.49^{f}	26.61±1.98^{k}
I. batatas	422.60±1.01^{b}	4.45±0.22^{d}	10.11±0.75^{cd}	120.60±0.20^{e}	21.82±0.71^{b}	152.53±1.67^{g}
M. esculenta	376.79±34.90^{c}	0.82±0.04^{g}	15.25±1.52^{b}	183.92±10.98^{b}	33.21±3.63^{d}	232.38±13.10^{b}
M. arboreus	274.46±10.34^{f}	16.86±1.62^{a}	2.35±0.41^{f}	46.43±5.85^{h}	7.38±1.10^{f}	56.16±7.35^{j}
S. melongena	354.87±32.56^{cd}	5.07±0.36^{d}	16.20±1.48^{b}	166.93±2.78^{c}	32.51±0.56^{b}	215.64±1.86^{c}
T. triangulare	205.50±10.51^{g}	11.08±0.28^{b}	11.41±1.38^{c}	123.51±1.42^{e}	32.25±1.47^{b}	167.17±1.34^{f}

Data are presented as means of triplicate analyses ± SD. Means with the same superscript letter in the same column for a single vegetable are not different at P > 0.05

3.2 Phenolics Profile and Antioxidant Activity

Total phenolics contents of selected leafy vegetables are depicted in Fig. 3. The values ranged from 179.66 ± 11.33 mg/100g dw in *C. olitorius* to 436.48 ± 1.73 mg/100g dw in *A. esculentus*. Phenolics are the main dietary antioxidants of vegetables and they include phenolic acids, flavonoids, tannins, coumarins, stilbenes and lignins [27]. Based on the chromatographic profile (Fig. 4), 3 phenolic acids (gallic acid, caffeic acid, chlorogenic acid) and 3 flavonoids (quercetin, catechin, kaempferol) were identified in the studied leafy vegetables.

Gallic acid belonging to hydroxybenzoic acids was only detected and quantified in 4 leafy vegetables (*B. alba*, *M. esculenta*, *M. arboreus* and *T. triangulare*) with highest content (25.37 ± 0.16 µg/g dw) for *M. esculenta* as indicated in the Table 3. It's important recalling that gallic acid is known to possess antimicrobial activity against human pathogens and this molecule can also scavenge electrophilic mutagens [28]. The non-detectable characteristic of gallic acid may be due to the fact that hydroxybenzoic acids contents of edible plants are generally low contrary to hydroxycinnamic acids (p-coumaric, caffeic, ferulic, chlorogenic and sinapic acids) [29]. Considering caffeic and chlorogenic acids

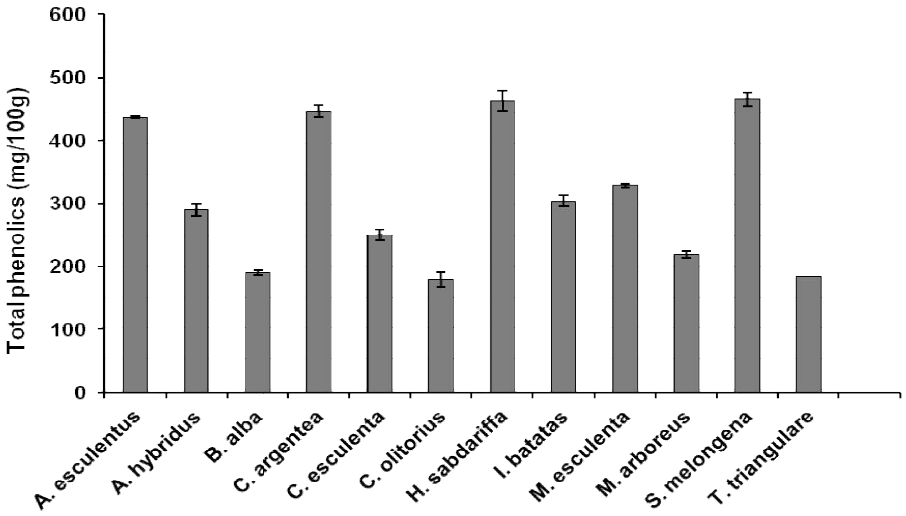

Fig. 2. Total phenolics of selected tropical leafy vegetables

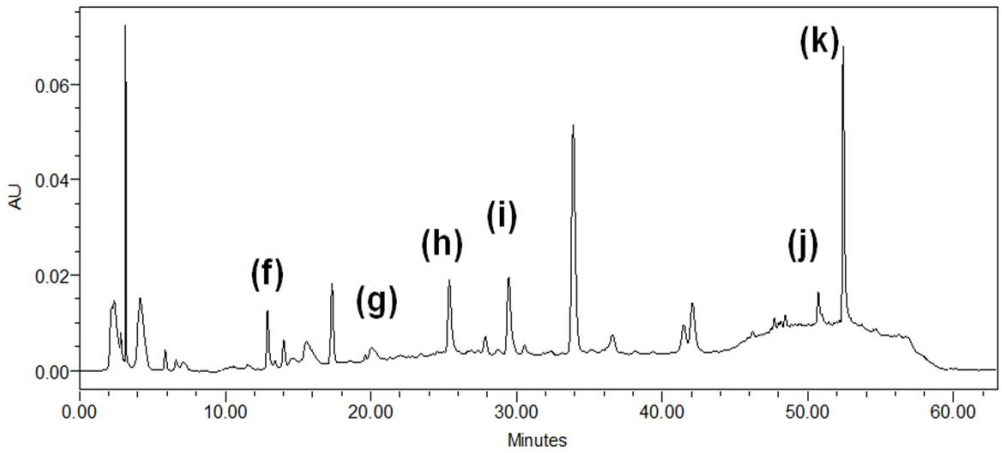

Fig. 3. Chromatrogram of phenolics from selected tropical leafy vegetables
(f): gallic acid; (g): catechin; (h): chlorogenic acid; (i): caffeic acid; (j): quercetin; (k) kaempferol

M. arboreus (7.08 ± 0.16 µg/g dw) and *I. batatas* (13.79 ± 0.51 µg/g dw) were respectively the leafy vegetables of interest. Especially for *I. batatas* leaves, the result above is not surprising since sweet potato tubers are known as rich sources of chlorogenic acid [30]. Furthermore, the content of chlorogenic acid in *I. batatas* leaves is higher than those (1.59 and 2.03 µg/g) of salad spinach and pak choi used as leafy vegetables in Western diet [31]. Based on anti-inflammatory and antioxidant activities of chlorogenic acid, increasing consumption of *I. batatas* leaves could help prevent diseases such as diabetes, obesity and cancer [32]. In addition to phenolic acids, quercetin (0.79 – 8.36 µg/g dw), catechin (0.39 – 5.65 µg/g dw) and kaempferol (0.76 – 29.11 µg/g dw) known as important flavonoids were also quantified in the selected leafy vegetables. this result corroborated the findings that flavonoids such as quercetin, kaempferol, myricetin and luteolin have been reported in leafy vegetables by some authors [33]. However, the contents of quercetin and kaempferol recorded in our study are lower than those (42 – 390 µg/g) of cabbages considered as rich sources of flavonols [34,35]. Flavonoids are known to possess many biological effects but the best described property is their antioxidant activity [36]. This antioxidant activity include multiple mechanisms such as scavenging reactive oxygen species (ROS), activation of antioxidant enzymes, metal chelating activity, inhibition of oxidases and reduction of α-tocopheryl radicals [37].

Table 3. Phenolics composition (µg/g dw) of selected tropical leafy vegetables

Leafy vegetables	Gallic acid	Caffeic acid	Chlorogenic acid	Quercetin	Catechin	Kaempferol
A. esculentus	nd	5.50±0.54^{b}	17.01±0.23^{a}	4.02±0.33^{d}	2.05±0.28^{c}	2.48±0.08^{d}
A. hybridus	nd	2.03±0.22^{e}	4.01±1.56^{e}	3.77±0.36^{d}	2.13±0.05^{c}	3.21±1.03^{d}
B. alba	8.03±1.23^{c}	nd	nd	0.98±0.01^{g}	1.48±0.09^{d}	0.76±0.03^{h}
C. argentea	nd	1.49±0.14^{f}	3.29±0.18^{f}	2.19±0.28^{f}	0.41±0.07^{f}	12.01±0.77^{b}
C. esculenta	nd	2.09±0.14^{e}	1.26±0.09^{i}	2.86±0.36^{e}	0.46±0.08^{f}	1.21±0.10^{f}
C. olitorius	nd	2.85±0.03^{d}	2.17±0.21^{h}	2.91±0.03^{e}	1.21±0.08^{e}	1.97±0.06^{f}
H. sabdariffa	nd	0.86±0.03^{g}	0.94±0.01^{j}	2.90±0.05^{e}	3.86±0.10^{b}	5.07±0.45^{c}
I. batatas	nd	3.96±0.07^{c}	13.79±0.51^{b}	8.36±0.10^{a}	0.48±0.07^{f}	3.34±0.88^{d}
M. esculenta	25.37±0.16^{a}	0.78±0.07^{g}	5.00±0.28^{e}	4.13±0.15^{c}	0.70±0.01^{f}	2.80±0.58^{e}
M. arboreus	4.65±0.92^{d}	7.08±0.16^{a}	8.53±0.13^{d}	0.79±0.01^{g}	5.65±0.15^{a}	1.04±0.16^{g}
S. melongena	nd	1.74±0.06^{f}	9.66±0.10^{c}	5.35±0.40^{b}	0.39±0.04^{g}	29.11±0.30^{a}
T. triangulare	13.81±0.21^{b}	nd	2.55±0.27^{g}	5.24±0.22^{b}	nd	nd

Data are presented as means of triplicate analyses ± SD. Means with the same superscript letter in the same column for a single vegetable are not different at P > 0.05. nd: non detected

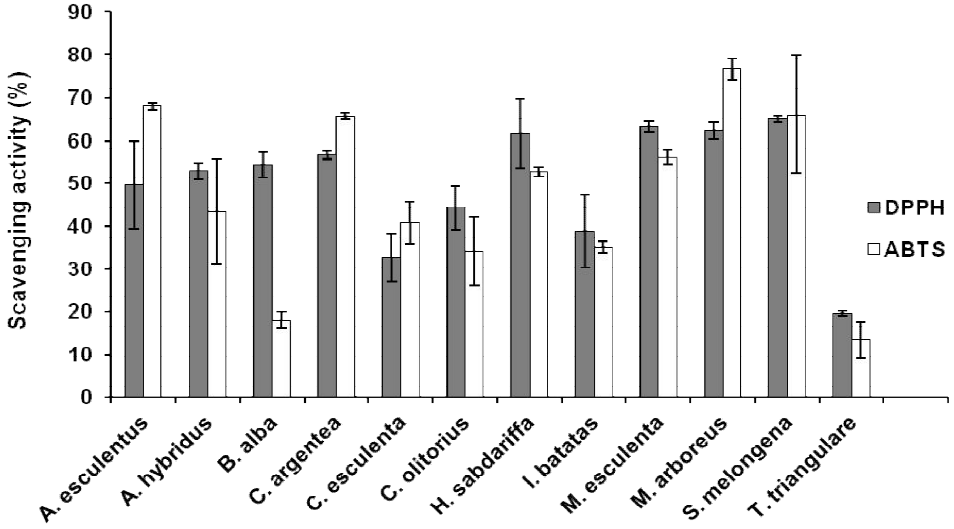

Fig. 4. Antioxidant activity of selected tropical leafy vegetables

In our study, antioxidant activity of selected leafy vegetables was evaluated by using DPPH and ABTS scavenging tests. These tests are based on electron transfer and they involve a reduction mechanism of a colored oxidant. Indeed, ABTS test is based on the reduction of blue/green ABTS cation radical while that of DPPH is based on the reduction of purple DPPH radical [38]. The values of DPPH and ABTS inhibition expressed in percentage are depicted by the Fig. 4. The DPPH scavenging activity of leaves ranged between 19.63 and 65% with *S. melongena* showing the highest value (65%). For ABTS, *M. arboreus* had the highest value (76.66%) of scavenging activity compared to other leafy vegetables. Antioxidant activity values found in our study were in range of 0.9 – 96% reported for African leafy vegetables [39]. The relatively high antioxidant activity values of *S. melongena* and *M. arboreus* leaves extracts indicated that they contained active radical scavengers that may protect cell tissues from damages, thereby preventing some diseases as cancer [40]. Regarding the values of antioxidant activity expressed as DPPH and ABTS inhibition percentage and phenolics contents, there was no direct correlation as previously demonstrated in some studies [41,42]. Apart from phenolics, other components such as carotenoids may also contribute to this antioxidant activity in the selected leafy vegetables.

4. CONCLUSION

The results obtained in this study show that the selected leafy vegetables contain appreciable amount of carotenoids and phenolics. Thus they can be considered as valuable sources of carotenoids and phenolics known as bioactives components. The studied tropical leafy vegetables could therefore contribute to the human health improvement and should be used as a source of nutrients to supplement other major diets. However, it is necessary to consider other aspects such as the bioavailability of these nutrients and the effect of various processing methods on nutritive value of these leafy vegetables.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Liu RH. Health-Promoting Components of Fruits and Vegetables in the Diet. Adv Nutr An Int Rev J. 2013;4(3):384S–392S.
2. Andarwulan N, Kurniasih D, Apriady RA, Rahmat H, Roto A V., Bolling BW. Polyphenols, carotenoids, and ascorbic acid in underutilized medicinal vegetables. J Funct Foods. 2012;4(1):339–47.
3. Liu RH. Potential synergy of phytochemicals in cancer prevention: mechanism of action. J Nutr. 2004; 134(12 Suppl):3479S–3485S. [Cited 2017 Sep 1]
4. Ambriz-Perez DL, Leyva-Lopez N, Gutierrez-Grijalva EP, Heredia JB. Phenolic compounds: Natural alternative in inflammation treatment. A review. Cogent Food Agric. 2016;2(1).
5. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci. 2007;8(9):950.
6. Kang NJ, Shin SH, Lee HJ, Lee KW. Polyphenols as small molecular inhibitors of signaling cascades in carcinogenesis. Pharmacol Ther. 2011;130(3):310–24.
7. Pérez-Jiménez J, Neveu V, Vos F, Scalbert A. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. Eur J Clin Nutr. 2010;64:S112–20.
8. Olson JA, Krinsky NI. Introduction: the colorful, fascinating world of the carotenoids: Important physiologic modulators. FASEB J. 1995;9(15):1547–50.
9. Nahak G, Suar M, Sahu RK. Antioxidant potential and nutritional values of vegetables: A Review. Res J Med Plant. 2014;8(2):50–81.
10. Mezzomo N, Ferreira SRS. Carotenoids functionality, sources, and processing by supercritical technology: A review. J Chem; 2016.
11. Fiedor J, Burda K. Potential role of carotenoids as antioxidants in human health and disease. Nutrients. 2014;6(2): 466–88.

12. Bakan E, Akbulut ZT, İnanc AL. Carotenoids in Foods and their Effects on Human Health. Acad Food J. 2014;12(2): 61–8.
13. Tanumihardjo SA. Carotenoids and human health. Carotenoids Hum Heal. 2013;55: 1–331.
14. Food and Agriculture Organization of the United Nations. Promotion of Fruit and Vegetables for Health; 2015.
15. Guarino L, International Plant Genetic Resources Institute. Traditional African vegetables : Proceedings of the IPGRI International Workshop on Genetic Resources of Traditional Vegetables in Africa: Conservation and Use, 29-31 August, 1995.
16. Maseko I, Id TM, Tesfay S. African Leafy Vegetables : A review of status. Production and Utilization in South Africa. 2017;1–16.
17. Tanumihardjo SA. Vitamin A: Biomarkers of nutrition for development. Am J Clin Nutr. 2011;94(2):658–65.
18. Institute of Medicine (U.S.). Panel on Micronutrients. DRI : Dietary reference intakes for vitamin A, vitamin K, arsenic, boron, chromium, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium, and zinc. Food and Nutrition Board, Institute of Medicine. National Academy Press. 2001;773.
19. Hertog MGL, Venema HPCH. Optimization of a Quantitative HPLC Determination of Potentially\rAnticarcinogenic Flavonoids in Vegetables and Fruits. J Agrlc Food Chem. 1992;40(9):1591–8.
20. Singleton VL, Orthofer R, Lamuela-Raventós RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. Methods Enzymol. 1999;299:152–78.
21. Choi CW, Kim SC, Hwang SS, Choi BK, Ahn HJ, Lee MY, Park SH, Kim SK. Antioxidant activity and free radical scavenging capacity between Korean medicinal plants and flavonoids by assay-guided comparison. Plant Sci. 2002; 163(6):1161–8.
22. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 1999;26(9–10):1231–7.
23. Rodriguez-amaya DB. A guide to carotenoids analysis. 2001;64.
24. Abdel-Aal ESM, Akhtar H, Zaheer K, Ali R. Dietary sources of lutein and zeaxanthin carotenoids and their role in eye health. Nutrients. 2013;5(4):1169–85.
25. Moeller SM, Jacques PF, Blumberg JB. The potential role of dietary xanthophylls in cataract and age-related macular degeneration carotenoids in the lens. 2000;19(5):522–7.
26. Wirth JP, Petry N, Tanumihardjo SA, Rogers LM, McLean E, Greig A, Garrett GS, Klemm RDW, Rohner F. Vitamin a supplementation programs and country-level evidence of vitamin A deficiency. Nutrients. 2017;9(3):1–18.
27. Naczk M, Shahidi F. Extraction and analysis of phenolics in food. J Chromatogr A. 2004;1054(1–2):95–111.
28. Karamaæ M, Kosiñska A, Pegg RB. Content of gallic acid in selected plant extracts. J Food Nutr Sci. 2006;15(1):55–8.
29. Pandey K, Rizv S. Plant polyphenols as dietary antioxidants in human health and disease. Oxid Med Cell Longev. 2009;2(5): 270–8.
30. Zheng W, Clifford MN. Food chemistry profiling the chlorogenic acids of sweet potato (*Ipomea batatas*) from China. 2008; 106:147–52.
31. Khanam UKS, Oba S, Yanase E, Murakami Y. Phenolic acids, flavonoids and total antioxidant capacity of selected leafy vegetables. J Funct Foods. 2012; 4(4):979–87.
32. Tajik N, Tajik M, Mack I, Enck P. The potential effects of chlorogenic acid, the main phenolic components in coffee, on health: A comprehensive review of the literature. Eur J Nutr. 2017;56(7):2215–44.
33. Trichopoulou A, Vasilopoulou E, Hollman P, Chamalides C, Foufa E, Kaloudis T, et al. Nutritional composition and flavonoid content of edible wild greens and green pies: a potential rich source of antioxidant nutrients in the Mediterranean diet. Food Chem. 2000;70(3):319–23.
34. Rochfort SJ, Imsic M, Jones R, Trenerry VC, Tomkins B. Characterization of Flavonol Conjugates in Immature Leaves of Pak Choi [*Brassica rapa* L. Ssp. *chinensis* L. (Hanelt.)] by HPLC-DAD and LC-MS/MS. J Agric Food Chem. 2006; 54(13):4855–60.
35. Vallejo F, Tomás-Barberán FA, García-Viguera C. Potential bioactive compounds in health promotion from broccoli cultivars

grown in Spain. J Sci Food Agric. 2002; 82(11):1293–7.
36. Shashank K, Pandey AK. Chemistry and biological activities of flavonoids. Hindawi Sci World J. 2013;2013(12):533–48.
37. Procházková D, Boušová I, Wilhelmová N. Antioxidant and prooxidant properties of flavonoids. Fitoterapia. 2011;82(4): 513–23.
38. Floegel A, Kim DO, Chung SJ, Koo SI, Chun OK. Comparison of ABTS/DPPH assays to measure antioxidant capacity in popular antioxidant-rich US foods. J Food Compos Anal. 2011;24(7):1043–8.
39. Uusiku NP, Oelofse A, Duodu KG, Bester MJ, Faber M. Nutritional value of leafy vegetables of sub-Saharan Africa and their potential contribution to human health: A review. J Food Compos Anal. 2010;23(6): 499–509.
40. Gunathilake KDPP, Ranaweera KKDS. Antioxidative properties of 34 green leafy vegetables. J Funct Foods. 2016;26:176–86.
41. Dasgupta N, De B. Antioxidant activity of some leafy vegetables of India: A comparative study. Food Chem. 2007; 101(2):471–4.
42. Ismail A, Marjan ZM, Foong CW. Total antioxidant activity and phenolic content in selected vegetables. Food Chem. 2004; 87(4):581–6.

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