A Comparative Study on Antioxidant Potential of Selected African and Exotic Leafy Vegetables

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Abstract. African leafy vegetables (ALVs) are thought to contain an immense variety of antioxidants, which may provide nutritional and health benefits. However, there is limited, robust, and comparable information describing their nutritional composition. The aim of this study is to determine the antioxidant potential of selected ALVs [black jack (*Bidens pilosa* L.) and amaranth (*Amaranthus hybridus* L.)] in comparison with exotic leafy vegetables (ELVs) [lettuce (*Lactuca sativa* L.), green cabbage (*Brassica oleracea*), red cabbage (*B. oleracea* var. *capitata*)]. Different nonenzymatic and enzymatic antioxidant assays were used to determine plant carbohydrates (CHOs) using high-performance liquid chromatography (HPLC), phenols (Folin–Ciocalteu), ascorbic acid, carotenoids, total antioxidant capacity (TAOC) using reducing ferric ion antioxidant potential (FRAP) and 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) assays, superoxide dismutase (SOD), catalase (CAT), and peroxidase (POD). Both amaranth and black jack produced high levels of CHOs, carotenoids, ascorbic acid, and phenols during early vegetative growth [4 weeks after transplanting (WAT)]. Similarly, SOD, CAT, and POD activity for amaranth and black jack also increased during early vegetative development. Conversely, exotic vegetables (lettuce and cabbages) only increased these antioxidants toward maturity. Overall, ALVs have produced higher concentrations of antioxidants specifically during early vegetative growth stage than the exotic vegetables. The promotion of ALVs could promote a healthy alternative especially in poor rural households.

Commercially available leafy vegetables are known for their nutritional properties, mostly rich in antioxidants (Shukla et al., 2006). Consuming these leafy vegetables that contain various antioxidant compounds may therefore boost the immune system. Consumers' awareness and interest on leafy vegetables has been increasing in recent times due to their perceived health image. However, the market for leafy vegetables is mainly dominated by ELVs, which is often unaffordable to poor rural communities. However, within these rural landscapes exists a rich diversity of ALVs, which has been relegated to the status of underused due to the promotion of ELVs. As such, the promotion of ALVs becomes a strategic solution to tap into their biodiversity for crop improvement

as well as to contribute to food and nutritional security, especially among infants, children, and pregnant and lactating women. Promoting cultivation of ALVs would increase their availability and accessibility to consumers and possibly generate household income for rural households.

ALVs represent a class of underused plants that are reported to be rich sources of natural antioxidants (Uusiku et al., 2010). Leafy vegetables also produce various types of antioxidants at different concentrations during their growing period (Moyo et al., 2013). From a plant physiology perspective, the purpose of antioxidants is to scavenge reactive oxygen species (ROS), most commonly hydrogen peroxide, the superoxide radical, and the hydroxide radical (Vranová et al., 2002), produced within the plant cell due to oxidative stress, before these oxidize vital cell components. Therefore, the occurrence of damaging concentrations of ROS needs to be counteracted to avoid permanent tissue damage (Seifried et al., 2007). Such an accumulation of ROS in the cell can be counteracted by a system of low molecular mass nonenzymatic antioxidants as well as

by certain enzymes, SOD, CAT, and PODs, which catalyze the destruction of ROS resulting in the production of nondamaging oxygen-containing compounds (Blokina et al., 2003). As such, ALVs' reported high levels of antioxidants make them suitable for cultivation in marginal production areas, which are often characterized by environmental stresses such as drought and heat stress.

ELVs have long been recognized as abundant sources of nutritious essential nutrients (Aletor et al., 2002; Shukla et al., 2006). They produce nutritious low molecular mass non-enzymatic, enzymatic antioxidants such as beta carotene, ascorbic acid, folic acid and riboflavin, SOD, CAT, APX, as well as minerals such as iron, calcium, and phosphorous (Monero et al., 2010). The vegetables also contain an immense variety antioxidants and phytochemicals, which provide health benefits beyond basic nutrition. Antioxidants' vitamins such as ascorbic acids and phenols are important in human nutrition because they function as an anticancer, anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, antibacterial, or antiviral agent (Dai and Mumper, 2010). Although the potential of exotic vegetables is widely known and reported on, the opposite is true for ALVs where evidence of their nutritional potential currently remains anecdotal.

ALVs, although widespread in rural landscapes, remain underused due to insufficient information on their production and nutrition. As such, their potential to contribute to food and nutritional security within vulnerable rural households remains untapped. ALVs, which can be free if harvested from the wild, and have low production costs could provide an affordable healthy alternative for rural households. Moyo et al. (2013) reported that in contrast to exotic vegetable, little attention had been paid to the production of ALVs and there was a dearth of information on their nutritional components, mainly antioxidants. The plant antioxidant distribution at different plant growth stages is also unclear. Thus, the current study aimed to investigate plant antioxidants for black jack (*B. pilosa*), amaranth (*A. hybridus*) in comparison with green cabbage (*B. oleracea*), red cabbage (*B. oleracea* var. *capitata*), and lettuce (*L. sativa*).

Materials and Methods

Materials. *Amaranthus hybridus* and *B. pilosa* seeds were harvested from the university farm, Ukulinga (29°37'S; 30°16'E; 775 m above sea level) and Agricultural Research Council (ARC-VOPI-South Africa), respectively. *Brassica oleracea*, *B. oleracea* var. *capitata* f. *rubra*, and *L. sativa* seeds were obtained from a local seed company [McDonald Seeds (Pty) Ltd]. All chemicals used for the experiment were all analytical grade, were obtained from Abcam®, Amersham®, Fluka®, Saarchem®, or Sigma-Aldrich®. DPPH, perchloric acid, trolox, metaphosphoric acid, Folin–Ciocalteu, gallic acid,

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Table 1. Water-soluble nonstructural carbohydrates accumulation by African and exotic leafy vegetables across different weeks after transplanting (WAT).

Leafy vegetable species	Time (WAT)	mg·g ⁻¹ dry weight				
		Raffinose	Glucose	Fructose	Sucrose	Total sugars
Amaranthus (<i>Amaranthus hybridus</i>)	2	0.26 ± 0.02	2.60 ± 0.20	1.40 ± 0.04	3.60 ± 0.12	7.86 ± 0.10
	4	0.54 ± 0.18	3.20 ± 0.35	4.20 ± 0.50	4.80 ± 0.36	12.74 ± 0.35
	6	1.20 ± 0.62	2.90 ± 0.41	5.00 ± 0.31	8.40 ± 0.24	17.56 ± 0.40
	8	0.85 ± 0.11	1.80 ± 0.12	2.40 ± 0.22	6.60 ± 0.18	11.65 ± 0.16
	10	0.26 ± 0.09	2.10 ± 0.06	1.80 ± 0.27	5.20 ± 0.23	9.36 ± 0.16
	12	0.32 ± 0.06	2.50 ± 0.14	1.94 ± 0.08	3.20 ± 0.17	7.96 ± 0.11
Mean		0.57 B ± 0.18	2.5 CD ± 0.21	2.79 C ± 0.24	5.3 B ± 0.22	11.1 B ± 0.21
Black jack (<i>Bidens pilosa</i>)	2	1.40 ± 0.07	0.90 ± 0.004	1.20 ± 0.06	2.80 ± 0.09	6.3 ± 0.07
	4	4.20 ± 0.13	0.60 ± 0.003	2.40 ± 0.09	4.50 ± 0.21	11.7 ± 0.12
	6	5.30 ± 0.21	2.20 ± 0.60	3.10 ± 0.06	6.10 ± 0.54	16.7 ± 0.35
	8	2.80 ± 0.09	1.40 ± 0.08	1.50 ± 0.01	5.80 ± 0.23	11.5 ± 0.10
	10	3.10 ± 0.15	1.20 ± 0.04	1.30 ± 0.09	4.60 ± 0.38	10.2 ± 0.15
	12	2.90 ± 0.08	0.90 ± 0.009	1.70 ± 0.04	3.10 ± 0.14	8.6 ± 0.07
Mean		3.2 A ± 0.12	1.2 D ± 0.13	1.86 C ± 0.05	4.48 B ± 0.27	10.8 B ± 0.14
Green cabbage (<i>Brassica oleracea</i>)	2	nd	6.50 ± 0.12	5.40 ± 0.21	6.70 ± 0.68	18.6 ± 0.34
	4	nd	8.30 ± 0.41	7.40 ± 0.93	8.40 ± 0.85	24.1 ± 0.73
	6	nd	6.50 ± 0.36	8.50 ± 1.02	8.97 ± 1.23	23.97 ± 0.87
	8	nd	7.20 ± 0.37	7.20 ± 1.20	9.40 ± 1.53	23.8 ± 1.03
	10	nd	9.40 ± 0.99	8.60 ± 0.69	8.50 ± 1.20	26.5 ± 0.96
	12	nd	9.10 ± 1.23	9.20 ± 1.03	7.40 ± 0.98	25.7 ± 1.08
Mean			7.8 A ± 0.58	7.71 A ± 0.85	8.2 A ± 1.08	23.7 A ± 0.84
Red cabbage (<i>Brassica oleracea</i> var. <i>capitata</i>)	2	nd	7.70 ± 0.97	6.80 ± 0.81	8.60 ± 1.08	23.1 ± 0.95
	4	nd	7.10 ± 1.05	6.30 ± 0.23	6.60 ± 1.10	20 ± 0.79
	6	nd	8.60 ± 1.17	4.30 ± 0.09	7.20 ± 1.81	20.1 ± 1.02
	8	nd	7.30 ± 1.03	8.80 ± 1.30	8.70 ± 1.23	24.8 ± 1.19
	10	nd	6.10 ± 0.98	9.40 ± 1.51	9.20 ± 2.14	24.7 ± 1.54
	12	nd	5.80 ± 0.65	7.40 ± 1.30	8.40 ± 1.18	21.6 ± 1.04
Mean			7.1 BC ± 0.98	7.2 B ± 0.87	8.1 B ± 1.42	22.4 B ± 1.09
Lettuce (<i>Lactuca sativa</i>)	2	nd	4.90 ± 0.71	5.20 ± 0.82	6.60 ± 2.14	16.7 ± 1.22
	4	nd	6.60 ± 0.68	6.20 ± 0.99	8.90 ± 1.51	21.7 ± 1.06
	6	nd	6.70 ± 0.94	5.90 ± 0.62	9.80 ± 1.52	22.4 ± 1.03
	8	nd	8.20 ± 1.25	8.80 ± 2.1	8.50 ± 1.24	25.5 ± 1.53
	10	nd	7.20 ± 2.01	8.12 ± 0.91	7.20 ± 2.01	24.8 ± 1.64
	12	nd	7.90 ± 1.07	8.70 ± 1.13	8.20 ± 1.81	24.8 ± 1.34
Mean			6.91 A ± 1.11	7.15 A ± 1.10	8.2 A ± 1.71	22.5 A ± 1.30
F Prob. (5%)		<0.001**	<0.001**	<0.001**	<0.01*	<0.01*
LSD (5%)		5	16	9	12	35

LSD = least significant difference.

Values are means ±SD (n = 5); values followed by a different uppercase letter within a column are significantly different at $P < 0.05$; nd = not detected.

and 2,4-dinitrophenylhydrazine were the major ones for antioxidant determinations.

Vegetable growing condition. The experiment was conducted in a growth tunnel ($\approx 18^\circ\text{C}$ day/ 33°C night and 60% to 80% relative humidity) at the University of KwaZulu-Natal controlled environment research unit (CERU), Pietermaritzburg, South Africa. Initially, seeds were sown into polystyrene trays with 200 cells (each cell $28 \times 28 \times 36$ mm). Three seeds were sown per cell and thinned 2 weeks after sowing to leave the strongest seedling. After 6 weeks of seedling growth, uniform seedlings were transplanted to plastic pots of 200 mm diameter filled with a soil mixture having a proportion of pine bark:peatmoss:sand (1:1:2). This proportion was used throughout the experimental period. The standard pH of the growing medium was between 5.3 and 6.0.

Experimental design. For this experiment, randomized complete design was used. There were 24 pots per cultivar. In total, there were 120 pots for the experiment. Before starting of the experiment, the potted plants were conditioned for 1 week under the same environmental conditions to allow them to adjust from transplanting shock. Pots were irrigated using drip irrigation programmed

three times per day for 5 min. Nutrients were supplied once a week throughout irrigation with vita feed fertilizer [GROMORE 3:1:3 (34) COMPLETE]. Plants were 7 weeks old at the start of the experiment. Pots were included into the experimental design for destructive leaf sampling which were later used for biochemical analysis. Fully matured four leaf samples were collected fortnightly for six successive times in early hours of the day from CERU glasshouse, Pietermaritzburg. The collected leaf samples were put into cooler box and immediately transported to plant physiology laboratory, Pietermaritzburg. After the leaf samples were freeze-dried using freeze-dryer, they were ground into powder and stored at -70°C for further analysis.

Measurement of antioxidants. Antioxidant levels were first determined as “TAOC” using the FRAP assay (Benzie and Strain 1996). Briefly, for FRAP assay, an aliquot of the samples (30 μL) extracted with 1 N perchloric acid was mixed with 900 μL FRAP reagent solution and the absorbance was measured at 593 nm after 10 min. The TAOC was expressed as $\mu\text{mol FeSO}_4 \times 7\text{H}_2\text{O} \times \text{g dry weight (DW)}$ equivalent.

To gauge the presence of hydrophilic as well as lipophilic antioxidants, DPPH assay was also used to determine total antioxidant activity (Leong and Shui 2002). Briefly, an aliquot (40 μL) of the same perchloric acid extract was added to 3 mL of methanolic DPPH solution. The change in absorbance at 515 nm was measured at 30 min. The antioxidant capacity based on the DPPH free radical scavenging ability of the extract was expressed as $\mu\text{mol Trolox equivalents per gram of plant material on dry basis}$.

Nonstructural soluble CHOs. Sugars were determined using HPLC-refractive-index detector (RID) according to Liu et al. (1999), with slight modifications. Briefly, freeze-dried material (0.10 g) was mixed with 10 mL 80% (v/v) ethanol and homogenized for 1 min. Thereafter, the mixture was incubated in an 80°C water bath for 60 min to extract the soluble sugars. Subsequently, the mixture was kept at 4°C overnight. After centrifugation at 12,000 g_n for 15 min at 4°C , the supernatant was filtered through glass wool and taken to dry in a vacuum concentrator. Dried samples were re-suspended in 2 mL ultra-pure water, filtered through a 0.45 μm nylon filter and analyzed using an isocratic HPLC system equipped with a RID

Fig. 1. Mean total carotenoids concentrations in African leafy and exotic vegetables at different weeks after transplanting (WAT). Vertical bars represent $\pm$ SE (n = 5).

on a Phenomenex[®] column (Rezex RCM–Monosaccharide). The concentration of individual sugars was determined by comparison with authentic sugar standards.

Determination of ascorbic acid concentration. The ascorbic acid concentration was determined according to Böhm et al. (2006), with slight modifications. Briefly, 0.10 g sample was mixed with 5 mL 0.56 M metaphosphoric acid, vigorously shaken, centrifuged at 2988 g_n , and the supernatant transferred into a volumetric flask. This procedure was repeated twice and the combined extracts made up to 20 mL using 0.56 M metaphosphoric acid. Subsequently, 200 μ L of this extract were mixed with 300 μ L 0.3 M trichloroacetic acid, centrifuged at 17,212 g_n for 10 min. Five subsamples of the supernatant (300 μ L aliquots) were mixed with 100 μ L 2,4-dinitrophenylhydrazine reagent (0.013 M in 30% perchloric acid) and heated to 60 °C for 1 h and subsequently cooled in an ice bath for 5 min. Thereafter, 400 μ L 15.75 M sulfuric acid were added to the sample and the absorbance read at 520 nm after 20 min. The ascorbic acid concentration was calculated by comparison of the values obtained with an L-ascorbic acid standard curve.

Determination of free- and membrane-bound phenols. Phenols were determined according to Hertog et al. (1992), with slight modifications. Briefly, freeze-dried material (1.0 g) was mixed with 10 mL 99.8% (v/v) methanol and vortexed for 30 s. Thereafter, the mixture was shaken overnight at room temperature to extract the free phenols. Subsequently, the mixture was centrifuged, and supernatant were filtered through Whatman[®] no. 1 filter paper and the sample was again rinsed with 10 mL of solvent until color was

no longer released. And acid hydrolysis was also used for the remaining plant residue to efficiently release cell wall-bound phenols. Briefly, a 10 mL portion of acidified (2 M hydrochloric acid) 60% aqueous methanol was added to each sample and placed in an oven at 90 °C for 90 min exactly. Tubes were allowed to cool, and supernatants were filtered through a 0.45 μ m filter. The total phenols concentration was determined spectrophotometrically using Folin–Ciocalteu reagent at 750 nm using gallic acid monohydrate as standard and the total phenolics concentration expressed as “gallic acid equivalents (GAE).”

Total flavonoid content. The total flavonoid content was also determined using a method that was described by Eghdami and Sadeghi (2010), with slight modifications. Briefly, a methanolic plant extract (0.10 mL) was added into a glass container. Subsequently followed by adding 5% NaNO₂ (0.03 mL) and the reaction mixture was allowed to stand on a room temperature for 5 min. A 0.03 mL of 10% AlCl₃ was added and incubated for 6 min, after that 0.2 mL of 1 M NaOH was added to the solution. And the reaction mixture was finally diluted to 1 mL by distilled water. The absorbance of the reaction mixture was read at 510 nm, against methanol used as blank. The results were finally expressed as mg “GAE”/g DW.

Determination of total carotenoids. The carotenoid content of leaf was measured in 85% acetone extract of the freeze-dried sample according to Svjetlana and Kristina (2010). The optical density of the extract was measured at 453, 505, 645, and 663 nm.

Total soluble protein extraction. Total crude proteins were extracted according to

Kanellis and Kalaitzis (1992), with slight modifications. Frozen mesocarp dry tissue powder (1.0 g) was extracted in 5 mL 50 mM Tris–HCl buffer (pH 7.4 containing 0.2 M NaCl, 20 mM MgSO₄, 1 mM EDTA, 5 mM β -mercaptoethanol, 0.5 mM PMSF, 10 mM leupeptin, and 10% (v/v) glycerol). The mixture was homogenized and allowed to stand on ice for 15 min and then centrifuged at 20,000 g_n for 20 min. The supernatant containing crude protein extract was used for enzyme assays after being passed through Miracloth[®].

Total protein assay for determination of enzyme activity. The Bradford microassay was used to determine the protein content of the samples (Bradford, 1976). Bradford dye reagent was prepared by diluting the dye concentrate with distilled water 1:4. The dye (1.0 mL) was added to test tubes containing 20 μ L sample extract, mixed, and incubated at room temperature for 5 min. Samples were then read spectrophotometrically at 595 nm and the protein concentration determined by comparing results with a standard curve constructed using bovine serum albumin.

CAT activity assay. A method originally described by Beers and Sizer (1952) was used with slight modifications to determine CAT (EC 1.11.1.6) activity. The reaction solution (3.0 mL) contained 0.05 M potassium phosphate (pH 7.0), 0.059 M hydrogen peroxide, 0.1 mL enzyme extract, and 1.9 mL distilled water. To start the reaction, the mixture, in absence of enzyme extract, was incubated for 4 to 5 min to achieve temperature equilibration and to establish a blank rate. To this mixture, 0.1 mL diluted enzyme extract was added and the disappearance of H₂O₂ was followed spectrophotometrically every 20 s for 3 min via the decrease in absorbance at 240 nm. The change in absorbance (Δ 240 nm·min⁻¹) from the initial (20 s) linear portion of the curve was calculated. One unit of CAT activity was defined as the amount that decomposes 1 μ mol H₂O₂. Enzyme activity was reported as units/mg protein using the following equation: units/mg protein = (Δ 240/min $\times$ 1000) $\times$ (43.6 $\times$ mg enzyme/mL of reaction mixture)⁻¹.

SOD activity assay. SOD (EC 1.15.1.1) activity was determined by measuring the ability of the enzyme to inhibit the photochemical reduction of nitro blue tetrazolium chloride (NBT), as described by Giannopolitis and Ries (1977). The reaction solution (3.0 mL) contained 1.5 mM NBT, 0.12 mM riboflavin, 13 mM methionine, 0.1 M EDTA, 67 mM phosphate buffer (pH 7.8), and contained 10 to 100 μ L enzyme extract. Riboflavin was added last and tubes were shaken and placed under fluorescent lighting with an intensity 8.42 μ mol·m⁻²·s⁻¹. Blanks and controls were determined by withholding illumination and without addition of enzyme, respectively. The absorbances of the illuminated and nonilluminated solutions were determined spectrophotometrically at 560 nm. One unit of SOD activity was defined as the amount of enzyme inhibiting 50% NBT photoreduction. The results were expressed

Fig. 2. Mean total phenols and flavonoids concentrations in African leafy and exotic vegetables at different weeks after transplanting (WAT). Vertical bars represent $\pm$ SE (n = 5).

as units $\times$ (mg protein) $^{-1}$ = 1000 $\times$ (μ g enzyme extract resulting in $\frac{1}{2}$ maximum inhibition) $^{-1}$.

Peroxides activity assay. POD (EC 1.11.1.7) activity was assayed according to Reuveni et al. (1992) by measuring the oxidation of guaiacol as the increase in absorbance at 470 nm from 0 to 3 min. The reaction mixture contained 15 mM of phosphate buffer (pH 6.0) and 100 μ L enzyme extract in a total volume of 1.0 mL. The reaction was initiated by adding 500 μ L substrate, which consisted of a 30 mM H_2O_2 and 80.7 mM guaiacol solution (o-methoxy-phenol). The POD activity was calculated as the change in optical density over a 3-min period and expressed as units per milligram of protein: units/mg protein = ($\Delta 470/\text{min} \times 1000$) $\times$ ($26.6 \times \text{mg enzyme/mL of reaction mixture}$) $^{-1}$.

Statistical analysis. Analyses of variance between vegetables and growing stage were performed using GenStat[®] version 17.1 (VSN International, UK). Standard deviation

values were calculated where a significant variance was found at $P < 0.05$ between individual values.

Results and Discussion

In this study, plant CHO accumulation varied significantly ($P < 0.05$) during the vegetative growth stage. Amaranth and black jack CHO concentrations displayed an increasing trend during the vegetative growth at 4 WAT (12.74 ± 0.35 mg/g DW; 11.70 ± 0.11 mg/g DW, respectively). The CHO content continued to increase until 6 WAT (17.56 ± 0.40 mg/g DW; 16.7 ± 0.40 mg/g DW, respectively); the concentration then declined toward maturity (Table 1). Out of the detected CHOs, green cabbage (142.67 ± 0.84 mg/g DW) and lettuce (136.1 ± 1.30 mg/g DW) had more total sugar concentrations. On the contrary, exotic vegetables' CHO concentration showed an increasing trend with plant age, green cabbage with highest concentration at 10 WAT (26.5 ± 0.96 mg/g

DW), red cabbage at 8 WAT (24.8 ± 1.19 mg/g DW), lettuce at 8 WAT (25.5 ± 1.53 mg/g DW). It is noticed that ALVs accumulated more CHOs during early plant development stages than exotic vegetables. On the contrary, exotic vegetables increased CHOs toward plant maturity stage. Our finding is found in agreement with Myczkowski et al. (1991), who reported exotic vegetables' CHO accumulation increases as plant matures. High leaf growth rate with increasing plant leaf area index increases plant efficiency by accumulating photoassimilates dominantly CHOs. The fact that ALVs showed high CHO levels during early growth means that they can be used from an earlier stage while exotic ELVs can only be used at maturity. This makes ALVs more suitable for piecemeal harvesting and plugging household food insecurity that usually occurs in between subsequent harvests.

Exceptionally, raffinose, a sugar alcohol was only detected for ALVs. Raffinose has antioxidant properties and can function as an osmotic regulator within plant cells; osmotic regulation is key to drought tolerance. The ability of ALVs to accumulate sugar alcohols, possibly owing to their adaptation to wild environments, allows them to adapt to drought conditions (Nishizawa-Yokoi et al., 2008). This emphasizes their suitability for cultivation in marginal production areas, which typify the rural areas especially under current and predicted effects of climate change (Alemayehu et al., 2014; Chivenge et al., 2015).

Plant total carotenoids were significantly different ($P < 0.05$) for all leafy vegetables (Fig. 1). Black jack had the highest concentration of total carotenoids at 4 WAT (4.2 ± 0.04 mg/100 g DW), which later decreased as the plants progressed toward maturity. On the contrary, concentration in amaranth increased with plant age and reached peak at 10 WAT (3.6 ± 0.4). The green, red cabbages and lettuce also showed similar trends with respect to carotenoid concentration (Fig. 1). It is noticed that the studied vegetables had wide variations in accumulating carotenoids during early plant developmental stages. And their variations in carotenoids narrowed as approaching toward maturity. Carotenoids, an antioxidant, protect plant cells from photooxidative damage (Britton et al., 1995, 1998) by scavenging excess ROS produced in the reaction center of photosystem II (Telfer, 2005). For all studied vegetables, plant carotenoid production at different development stages may be specific to these cultivars. This observation is consistent with Reif (2012) who reported that biochemical composition of plants, especially carotenoids, is cultivar specific.

Human consumption of carotenoids is crucial to their nutrition and health. Carotenoids are precursors of vitamin A (retinal), which plays a huge role for vision, and retinoic acid, which plays a role in cellular differentiation (Bartley and Scolnik, 1995). Therefore, a diet rich in carotenoids can contribute to reduced oxidative damage

Fig. 3. Mean total ascorbic acid concentrations in African leafy and exotic vegetables at different weeks after transplanting (WAT). Vertical bars represent $\pm$ SE ($n = 5$).

(Rao and Rao, 2007; Von Lintig, 2010), associated with several pathologies, including aging (Esterbauer et al., 1992) and carcinogenesis (Breimer, 1990). The results of this study highlighted that ALVs had comparable levels of carotenoids with ELVs. This is important for promoting them as healthy alternatives alongside the already established ELVs.

Plant phenolic content varied significantly ($P < 0.05$) for all vegetables and at different plant developmental stages (Fig. 2). For black jack, total phenol concentration increased during the early stages of plant development, from 280 ± 12.1 mg/100 g DW at 2 WAT to 360 ± 10.60 mg/100 g DW at 4 WAT. Thereafter, the concentration decreased sharply as the plants matured. Similarly, amaranth's phenols increased from 150 ± 8.4 mg/100 g DW at 4 WAT and reached peak to 320 ± 6.8 mg/100 g DW at 6 WAT before decreasing toward plant maturity. Although ELVs also produced phenols, the levels were increasing up till maturity implying that they are most nutritious at maturity. It is also noticed that the studied vegetables had wide variations in accumulating total phenols during early plant developmental stages, which narrowed toward maturity. Dumas et al. (2003) also observed that commercial vegetables' phenols tended to increase during their development and only scored maximum concentration when approaching to plant maturity. Biosynthesis of plant phenolics is mainly triggered by

biotic and abiotic factors. Accumulation of metabolites often occurs in plants subjected to stresses including various elicitors or signal molecules. The fact that ALVs started with high levels of phenols suggests that they are adapted to establishing under harsh conditions. This would confer upon them a degree of stress tolerance during early establishment.

From a nutritional perspective, it could be speculated that consumption of young black jack leaves may be associated with less bitterness compared with consuming fully mature leaves. This is because phenolic compounds influence bitterness and astringency as well as the overall quality of a plant (Rahman et al., 2007). Plant phenols also contribute to specific odors, tastes, and colors in plants (Spencer et al., 2008). Plant foods that are rich sources of phenolics have been reported to prevent cardiovascular diseases, reduce inflammation, lower the incidence of cancers and diabetes, as well as reduce rates of mutagenesis in human cells (Scalbert et al., 2005). The protection afforded by the consumption of plant products such as fruits, vegetables, and legumes is mostly associated with the presence of phenolic compounds. In this regard, consumption of ALVs would be beneficial to human nutrition and health in marginal production areas where ELVs may not be adapted to.

Plant ascorbic acid was significantly different ($P < 0.05$) among the leafy vegetables (Fig. 3). Both amaranth and black jack produced high ascorbic concentration during

early vegetative growth i.e., 84 ± 1.2 mg/100 g DW at 2 WAT and 83 ± 0.8 mg/100 g DW at 4 WAT, respectively (Fig. 3). Consistent with the trend observed for other phytochemicals, ascorbic acid concentration slowly declined with plant age. On the contrary and consistent with previous results of the current study, the ELVs showed an increasing trend in total ascorbic acid over time. It was noticed that the studied vegetables had wide variations in ascorbic acid during early plant developmental stages. And their variations narrowed as approaching toward maturity. The high level of ascorbic acid in young leaves of ALVs may be attributed to high glucose concentration which is the main precursor in the biosynthesis of L-ascorbic acid. This observation concurs with Dumas et al. (2003) observations in tomato leaves, whereby high ascorbic acid concentration was strongly correlated to high sugar concentration.

CAT activity for amaranth and black jack increased significantly during early vegetative stages and reached maximum concentration at 6 WAT (84.7 ± 0.62 U/mg/min) and 4 WAT (81.1 ± 1.6 U/mg/min), respectively (Table 2). For the ELVs, CAT activity showed a slight increase during the early growth for about 8 WAT and decreased toward the end. The enzyme activity could have increased in response to high accumulation of H_2O_2 due to high plant metabolic rates. In leafy vegetables, CAT is required and contributes hugely in the early events of seedling growth, because it alleviates H_2O_2 produced during high rates of plant growth (Bailly et al., 2004).

Similar to results of CAT activity, SOD activity of black jack and amaranth increased significantly during early vegetative stages and peaked at 6 WAT (151.5 ± 1.89 U/mg/min) and at 4 WAT (162.8 ± 2.70 U/mg/min) for amaranth and black jack, respectively (Table 2). SOD activity of the ELVs only reached peak at 6 WAT for lettuce (85.2 ± 1.41 U/mg/min), and at 8 WAT for green cabbage (100 ± 1.65 U/mg/min) and red cabbage (85.1 ± 1.41 U/mg/min). The SOD mainly dismutates the super oxides into H_2O_2 ; the higher product of this ROS might be associated with increased SOD activity. This also confirms the holistic strength between SOD activity and CAT activity, the CAT activity depends on the H_2O_2 concentration which is the product of SOD (Mhamdi et al., 2010).

Earlier reports (Rogozhin et al., 2000) illustrate that increased SOD activities and cellular ROS levels were involved in the life of many plants including developmental course such as seed germination. Enhanced SOD activity can be triggered by increased production of ROS or it might be a protective measure adopted by plants against oxidative damage. Our findings, were also in line with Dučić et al. (2003) that SOD participated in the defense mechanism during early seedling development. The control of steady-state ROS levels by SOD is an important protective mechanism against cellular oxidative

Table 2. Enzymatic antioxidants (SOD, CAT, and POD) activities by ALVs and ELVs during plant growing time after WAT.

Leafy vegetable species	Time (WAT)	U/mg/min			Mean enzyme activity
		SOD	CAT	POD	
Amaranthus (<i>Amaranthus hybridus</i>)	2	120.1 ± 1.21	54.2 ± 0.75	20 ± 0.69	64.8 ± 0.88
	4	139.6 ± 2.1	78.2 ± 1.14	42.2 ± 0.71	86.7 ± 0.32
	6	151.5 ± 1.89	84.7 ± 0.62	45.1 ± 1.3	93.8 ± 1.27
	8	69.1 ± 1.5	45.2 ± 0.75	31.5 ± 0.47	48.6 ± 0.91
	10	27.1 ± 1.1	30.4 ± 0.42	23.6 ± 0.38	27 ± 0.63
	12	24.6 ± 1.3	27.1 ± 0.74	19.8 ± 0.64	23.8 ± 0.89
	Mean	88.7 AB ± 1.52	53.3 A ± 0.74	30.4 A ± 0.7	57.4 ± 0.98
Black jack (<i>Bidens pilosa</i>)	2	141 ± 2.1	65.1 ± 1.42	34.8 ± 0.83	80.3 ± 1.45
	4	162.8 ± 2.7	81.1 ± 1.6	46.2 ± 0.59	96.7 ± 1.63
	6	154.1 ± 2.51	70.9 ± 1.47	49.8 ± 0.86	91.6 ± 1.61
	8	89.1 ± 1.89	51.1 ± 2.1	32.1 ± 0.61	57.4 ± 1.53
	10	66.7 ± 1.20	42.3 ± 1.44	21.6 ± 0.68	43.5 ± 1.11
	12	64 ± 1.54	39.1 ± 0.98	14.6 ± 0.53	39.2 ± 1.02
	Mean	113 A ± 1.99	58.3 A ± 1.50	33.2 A ± 0.68	68.1 ± 1.4
Green cabbage (<i>Brassica oleracea</i>)	2	67.1 ± 0.43	24.2 ± 0.82	10 ± 0.16	33.8 ± 0.47
	4	81.4 ± 0.58	39.2 ± 1.2	19.5 ± 0.75	46.7 ± 0.84
	6	94.2 ± 1.38	58.9 ± 1.46	21.3 ± 0.85	58.1 ± 1.23
	8	100 ± 1.65	62.8 ± 0.62	27.4 ± 0.51	63.4 ± 0.93
	10	60.2 ± 1.1	19.7 ± 0.3	35.1 ± 0.49	38.3 ± 0.63
	12	56.4 ± 1.3	17.9 ± 0.38	15.4 ± 0.54	29.9 ± 0.74
	Mean	76.6 AB ± 1.07	37.1 AB ± 0.80	21.5 A ± 0.55	45 ± 0.81
Red cabbage (<i>Brassica oleracea</i> var. <i>capitata</i>)	2	50.6 ± 1.08	16.4 ± 0.68	19.4 ± 0.45	28.8 ± 0.74
	4	62.1 ± 0.41	32.4 ± 1.21	32.8 ± 0.36	42.4 ± 0.66
	6	69.4 ± 1.30	49.6 ± 0.64	35.6 ± 0.26	51.5 ± 0.73
	8	85.1 ± 1.41	50.0 ± 0.69	30.1 ± 0.81	55 ± 0.97
	10	50.1 ± 0.32	21.5 ± 0.41	28.4 ± 0.68	33.3 ± 0.47
	12	51 ± 0.84	18.7 ± 0.48	16.4 ± 0.65	28.7 ± 0.66
	Mean	61.4 B ± 0.89	31.4 B ± 0.69	21.1 A ± 0.54	40 ± 0.7
Lettuce (<i>Lactuca sativa</i>)	2	47 ± 1.51	26.7 ± 0.69	12.9 ± 0.64	28.8 ± 0.95
	4	67 ± 0.59	38.2 ± 0.62	22.1 ± 1.5	42.4 ± 0.90
	6	85.2 ± 1.41	42.9 ± 1.22	29.4 ± 2.3	52.5 ± 1.64
	8	77.2 ± 1.1	56.4 ± 1.5	33.4 ± 0.39	55.7 ± 1.0
	10	26 ± 0.55	33.8 ± 0.83	35.6 ± 0.23	31.8 ± 0.54
	12	24.1 ± 0.46	25.6 ± 0.68	13.5 ± 0.77	21.1 ± 0.64
	Mean	54.4 B ± 0.94	37.3 AB ± 0.92	24.5 A ± 0.97	38.7 ± 0.94
F prob. (5%)		0.06	0.06	0.34	
LSD (5%)		42.48	21.38	12.41	

ALV = African leafy vegetables; ELV = exotic leafy vegetables; CAT = catalase; LSD = least significant difference; POD = peroxidase; SOD = super oxide dismutase; WAT = weeks after transplanting.

Values are means ± sd (n = 5); values followed by a different uppercase letter within a column are significantly different at $P < 0.05$.

damage, since O_2^- acts as a precursor of more cytotoxic or highly relative ROS (Mittler et al., 2004). This reaffirms the trend of results implying greater adaptation to harsh environments in ALVs than ELVs. This suggests that ALVs may be useful for cultivation in harsh environments where ELVs may struggle to establish.

Consistent with CAT and SOD activity, black jack and amaranth displayed a trend of increasing POD activity during early vegetative stages. POD activity reached peak concentration at 6 WAT for both amaranth (45.1 ± 1.30 U/mg/min) and black jack (49.8 ± 0.86 U/mg/min) (Table 2). For the ELVs, POD activity reached peak at 6 WAT for red cabbage (35.6 ± 0.26 U/mg/min) and at 10 WAT for both green cabbage (35.1 ± 0.49 U/mg/min) and lettuce (35.6 ± 0.23 U/mg/min). POD has been reported (Blokhina et al., 2003) to work in collaboration with SOD and CAT to remove O_2^- and H_2O_2 , respectively. Moreover, Rogozhin et al. (2000) reported that the changes of POD activity in the earliest stage of plant development were correlated to those of SOD and CAT activities.

Diverse environmental stresses differentially affect plant processes that lead to loss of cellular homeostasis accompanied by the

formation of ROS, which causes oxidative damage to membranes, lipids, proteins, and nucleic acids (Srivalli et al., 2003). The coordinated function of antioxidant enzymes such as SOD, CAT, and POD helps in processing of ROS and regeneration of redox ascorbate and glutathione metabolites (Foyer and Nector, 2000; Halliwell, 1974; Wise, 1995). The oxidative damage to cellular components is limited under normal growing conditions due to efficient processing of ROS through a well-coordinated and rapidly responsive antioxidant system consisting of several enzymes and redox metabolites. The results of the current study imply that ALVs have a better coordinated response during early growth stages than ELVs.

The TAOC of the studied vegetables was determined using FRAP and DPPH assays. Since the DPPH assay results had no significant variation among the vegetables, we decided to use only FRAP result for discussion. Interestingly, the FRAP results showed an increasing trend for all vegetables (Table 3). The ALVs recorded the maximum concentration toward later stage of growth at 10 WAT for black jack (1651.45 ± 339.5 $\mu\text{moles}\cdot\text{g}^{-1}$ DW) and at 12 WAT for amaranth (5637.4 ± 104.6 $\mu\text{moles}\cdot\text{g}^{-1}$ DW).

ELVs recorded maximum concentration at 10 WAT for red cabbage (4349.8 ± 196 $\mu\text{moles}\cdot\text{g}^{-1}$ DW) and lettuce (2200 ± 386 $\mu\text{moles}\cdot\text{g}^{-1}$ DW) and at 12 WAT for green cabbage (4293.6 ± 53.1 $\mu\text{moles}\cdot\text{g}^{-1}$ DW). Total antioxidant potential could be impacted by various cellular nonenzymatic as well as enzymatic antioxidants during plant growing period. This was also confirmed by correlation analysis among different studied parameters (data not presented).

In conclusion, the present findings suggest that ALVs produce higher antioxidants compared with ELVs. On average, both amaranth and black jack produce higher levels of CHOs, carotenoids, ascorbic acid, phenols between 4 and 6 WAT. Conversely, the exotic vegetables produce these compounds as they approach harvest maturity. The ability of ALVs to produce high levels of antioxidants during early plant growth stages highlights their adaptation to harsh environments where conditions are usually unfavorable for early growth and development. Furthermore, their adaptation to such environments and high levels of nutrition make them ideally suitable to contribute to food and nutritional security of people residing on marginal agricultural production areas.

Table 3. Total antioxidant capacity (using DPPH and FRAP assays) by African and exotic leafy vegetables across different WAT.

Leafy vegetable species	Time (WAT)	$\mu\text{moles}\cdot\text{g}^{-1}$ dry weight	
		DPPH	FRAP
Amaranthus (<i>Amaranthus hybridus</i>)	2	5.41 $\pm$ 0.1	224.93 $\pm$ 28.8
	4	8.25 $\pm$ 0.3	211.78 $\pm$ 33.2
	6	9.04 $\pm$ 0.1	4,596.45 $\pm$ 174.5
	8	9.12 $\pm$ 0.1	3,246.32 $\pm$ 263.1
	10	8.02 $\pm$ 0.3	5,132.64 $\pm$ 116.2
	12	7.40 $\pm$ 0.2	5,637.36 $\pm$ 104.6
Mean		7.8 A $\pm$ 0.2	3,174.9 A $\pm$ 120
Black jack (<i>Bidens pilosa</i>)	2	5.36 $\pm$ 1	354.02 $\pm$ 51.1
	4	5.43 $\pm$ 0.8	387.25 $\pm$ 62.2
	6	5.49 $\pm$ 0.04	751.54 $\pm$ 195
	8	4.56 $\pm$ 0	968.21 $\pm$ 218
	10	4.58 $\pm$ 0	1,651.45 $\pm$ 339.5
	12	3.80 $\pm$ 0	1,000.2 $\pm$ 289.2
Mean		4.9 B $\pm$ 0.3	852.1 B $\pm$ 192.5
Green cabbage (<i>Brassica oleracea</i>)	2	5.31 $\pm$ 0.4	405.59 $\pm$ 125.0
	4	5.54 $\pm$ 0.1	374.17 $\pm$ 87.2
	6	5.38 $\pm$ 0.1	2,184.87 $\pm$ 121.0
	8	5.31 $\pm$ 0.3	2,504.55 $\pm$ 40.5
	10	5.20 $\pm$ 0.3	3,710.56 $\pm$ 120.8
	12	4.90 $\pm$ 0.2	4,293.6 $\pm$ 53.1
Mean		5.2 B $\pm$ 0.2	2,245.6 AB $\pm$ 91.3
Red cabbage (<i>Brassica oleracea</i> var. <i>capitata</i>)	2	5.11 $\pm$ 0.2	166.86 $\pm$ 107.2
	4	5.38 $\pm$ 0.1	473.49 $\pm$ 22.8
	6	5.26 $\pm$ 0.2	2,906.13 $\pm$ 81.3
	8	4.97 $\pm$ 0.2	2,805.02 $\pm$ 297.0
	10	4.80 $\pm$ 0.3	4,349.81 $\pm$ 196.0
	12	4.90 $\pm$ 0.3	3,588.06 $\pm$ 294.8
Mean		5.1 B $\pm$ 0.2	2,381.6 AB $\pm$ 166.5
Lettuce (<i>Lactuca sativa</i>)	2	5.37 $\pm$ 0.1	564.12 $\pm$ 48.6
	4	5.46 $\pm$ 0.12	398.12 $\pm$ 29.4
	6	5.36 $\pm$ 0.3	745.12 $\pm$ 110.0
	8	5.07 $\pm$ 0.02	1,400.23 $\pm$ 120.6
	10	4.76 $\pm$ 0.1	2,200 $\pm$ 386.0
	12	4.10 $\pm$ 0.2	1,800 $\pm$ 235.0
Mean		5.02 B $\pm$ 0.1	1,184.6 B $\pm$ 154.9
F prob (5%)		<0.001	0.052
LSD (5%)		0.871	1,854

DPPH = 1,1-diphenyl-2-picrylhydrazyl free radical; FRAP = ferric ion antioxidant potential; LSD = least significant difference; WAT = weeks after transplanting. Values are means $\pm$ SD (n = 5); values followed by a different uppercase letter within a column are significantly different at $P < 0.05$.

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