Minerals, trace elements and antioxidant phytochemicals in wild African dark-green leafy vegetables (*morogo*)

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Wild African dark-green leafy vegetables (*morogo*) are an important constituent of the traditional starch-based African diet. Three wild *morogo* types were sampled from different geographical regions in South Africa to determine their mineral, total polyphenol, total carotenoid and beta-carotene contents. Mineral and trace element compositions were determined using inductively coupled argon plasma mass spectrometry (ICP-MS). Concentrations of total carotenoids and total phenolics were measured by spectrophotometry and beta-carotene concentrations by high performance liquid chromatography (HPLC). In comparison with values reported for commercial spinach and swiss chard, results from the present study indicate relatively high calcium and magnesium concentrations in the wild *morogo*. Total carotenoid concentrations in the three *morogo* types were comparable with that of spinach. Beta-carotene concentrations were highest in *Amaranthus hybridus*, but this value was lower than those reported for other *morogo* species grown commercially. Concentrations of total phenolics were in the same range as those reported for conventional and commercially-grown non-conventional dark-green leafy vegetables. Results from the present study suggest that readily accessible wild *morogo* varieties represent inexpensive sources of dietary minerals, trace elements and antioxidant phytochemicals.

Key words: African dark-green leafy vegetables, *morogo*, minerals, trace elements, antioxidant phytochemicals

Introduction

The leaves of local wild and cultivated plants feature prominently as vegetables in the traditional starch-based African diet.[1] Some African green leafy vegetables are classified as functional foods because they have health benefits beyond basic nutrition.[2] The dietary value of plant leaves pertains to their position as primary producers in the human food chain. Green plants absorb the minerals they need for diverse anabolic processes from the soil. The complex organic compounds thus manufactured in plant leaves include antioxidant molecules for protection against oxidative solar radiation.[3] Leafy vegetables are, therefore, important dietary sources of minerals, trace elements and phytochemicals with health-protective and immune-strengthening properties. Molecular evidence suggests that trace elements and antioxidant molecules in green leafy vegetables lower the risks of cancer and cardiovascular diseases through mechanisms that modulate free radical attack on nucleic acids, proteins and polyunsaturated fatty acids.[4–6] Lako *et al.*[7] demonstrated that leafy vegetables have a higher antioxidant capacity than either fruits or root crops.

Setswana and Sesotho speakers use the term *morogo* collectively in reference to the aerial parts of edible plants consumed as green leafy vegetables.[8] Plants utilised as *morogo* are either indigenous or naturalised in the areas where they grow and many are accessible from the wild.[9] Traditional African dark-green leafy vegetables (DGLVs) are underutilised and neglected in areas where people consider them inferior to commercially-produced conventional vegetables of the westernised diet.[1] The general lack of information on the chemical composition, nutritional value and health benefits of traditional African DGLVs may also negatively influence perceptions of *morogo* consumption.

The present study reports on the mineral and phytochemical composition of wild-growing varieties of three types of *morogo* widely consumed in different geographical regions of South Africa. The mineral and trace elements contents, carotenoid and polyphenol levels in *morogo* were compared with those of conventionally- and commercially-grown DGLVs. The nutritional benefits and potential health-protective value of *morogo* consumption are briefly discussed.

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Materials and Methods

Sample collection and preparation

Sampling localities were situated in the Rustenburg district of the North West Province and Vhembe and Capricorn districts of the Limpopo Province of South Africa. These localities are considered low-rainfall areas with average annual rainfalls of 600 mm, 450 mm and 339 mm, respectively. Average temperature ranges for these areas are 3–31°C (Rustenburg), 7–34°C (Vhembe) and 6–26°C (Capricorn).[10] The veld surrounding the rural villages of Phokeng and Zuurplaat (Rustenburg), Nzelele Valley (Vhembe) and Dikgale (Capricorn) were searched, in the company of women from the community, for *thepe, lerotho* and *dinawa* – wild *morogo* types that are used by the households in those communities. Herbarium specimens of these plants were prepared and used for subsequent identification. *Thepe* was identified as *Amaranthus hybridus* L. subsp. *hybridus* and *Amaranthus thunbergii* Moq., *lerotho* as *Cleome gynandra* L. and *dinawa* as *Vigna unguiculata.*

Fresh leaves were picked from at least four different plants growing within an undetermined surface area. The number of leaves taken from each plant depended on the size of the plant. Upon collection, the leaves of the different plants were transferred to separate zip-lock plastic bags, transported to the laboratory on ice and freeze-dried immediately upon arrival at the laboratory. Freeze-dried samples were stored at –20°C until analysis. Finely ground freeze-dried samples were used in subsequent chemical analyses.

Chemicals

All standards were purchased from Sigma (U.S.A.). All organic solvents used were of HPLC grade, purchased from Burdick and Jackson (U.S.A.). All other reagents were purchased from Merck (Darmstadt, Germany).

Mineral and trace element analysis

A 100-mg freeze-dried sample was accurately weighed and carefully heated in 1 ml of nitric acid (70%) until clarity was achieved. After cooling, 3 ml of water was added and heating resumed for a further 10 min. Finally, the solution was cooled and deionised water was added until a volume of 10 ml was attained. The mineral composition of each sample was determined using an Agilent 7500c inductively coupled argon plasma mass spectrometer (ICP-MS). Calibrations were performed using external standards prepared from a 1 000 ppm single stock solution made up with 2% nitric acid. The external calibrations were run in the same analytical sequence as the samples. Three separate samples for each plant were analysed in this manner and values are reported as the mean ± standard deviation in mg 100 g^{-1} dry mass.

Total phenolics analysis

The extraction of total phenolics in each sample was carried out in triplicate, according to the modified method of Kähkönen *et al.*[11] Ground, dry plant material (25 mg) was extracted with 5 × 1 ml of 80% aqueous methanol using an Ultra Turrax mixer for 5 min. Samples were centrifuged at 3 000 rpm for 10 min and extracts were nitrogen dried. Residues were dissolved in 1 ml aqueous methanol (80%). The amount of total phenolics in extract samples was determined, as previously described,[12] using Folin-Ciocalteu's procedure.[13] Volumes of 200 μl of extract were transferred to test tubes and 1 ml Folin-Ciocalteu's reagent was added. The mixture was allowed to stand for 8 min at room temperature. A volume of 0.8 ml sodium carbonate (7.5%) was subsequently added, mixed and allowed to stand for 30 min. Absorption was measured at 765 nm (Shimadizu UV-1601 Spectrophotometer). Total phenolic content was expressed as mean ± standard deviation gallic acid equivalents (GAE) in mg 100 g^{-1} dry mass.

Total carotenoids analysis

Total carotenoids content for each plant sample was extracted and analysed in triplicate as decribed by Edwards *et al.*[14] with slight modifications. Approximately 25 mg of sample was weighed into a 10-ml centrifuge tube with 3 g of glass beads, then mixed with 5 ml dimethylsulphoxide (DMSO), and placed in a pre-heated water bath at 45°C for 30 min. The tube was vortexed for 15 s every 10 min. After incubation, samples were centrifuged at 4 000 rpm for 5 min. The supernatant was collected in a 25-ml volumetric flask. The samples were re-extracted with 5 ml acetone until the absorbance of the centrifuged layer was less than 0.005. Acetone was added to the collected supernatant such that a final volume of 25 ml was achieved. The absorbance was determined at 477 nm (Shimadizu UV-1601 spectrophotometer). Total carotenoid content is described as mean ± standard deviation in mg 100 g^{-1} dry mass.

Beta-carotene extraction and HPLC analysis

A modification of the procedure described by Lakshiminarayana *et al.*[15] was subsequently applied to 50 mg of each sample for extraction of beta-carotenes in triplicate. Extraction was carried out in triplicate in ice-cold acetone and the procedure was repeated until the extract was colourless. An aliquot of 1.5 ml of each extract was dried under a stream of nitrogen. The residue was redissolved in 250 μl of acetone before being filtered by a 0.45-μm nylon membrane filter. Sample filtrates were analysed by HPLC to determine the beta-carotene contents and reported as mean ± standard deviation in mg 100 g^{-1} dry mass.

High performance liquid chromatography analysis

Analytical separations were performed according to a modified procedure of De Ancos *et al.*[16] using a Hewlett-Packard System Series Model 1100 with a UV-visible detector.

The column was a 4.6 mm × 250 mm Nova-Pack C18 4 μm Waters (Waters Corporation). Solvents were HPLC-grade methanol and ethyl acetate. A gradient system involving two mobile phases was used. Mobile phase A consisted of methanol:water (75:25 v/v) and mobile phase B of ethyl acetate. A gradient was run starting from 0% B to 70% B in 10 min, followed by 70% B to 100% B in 4 min. Beta-carotene eluted at approximately 15 min. At the end of the gradient, the column was re-equilibrated under the initial conditions by a new gradient condition beginning at 14 min until 20 min, with a final composition of eluent B at 0%. Standard curves of beta-carotene (95%) were constructed by plotting HPLC peak absorbance area versus concentration of the beta-carotene in the injected sample.

Results

Mineral and trace elements (Table 1)

Calcium concentrations (mg 100 g^{-1} dry weight) in fresh samples ranged between 1 722 ± 60 (*V. unguiculata*) and 3 100 ± 95 (*C. gynandra*; Rustenburg). Calcium concentration in fresh *A. hybridus* (Rustenburg) was considerably higher than that of *A. hybridus* (Vhembe). Magnesium concentrations (mg 100 g^{-1} dry weight) ranked highest in the Rustenburg District samples of *A. hybridus* (1 400 ± 0.3) and *C. gynandra* (1 311 ± 45). *Amaranthus thunbergii* contained less magnesium (520 ± 18), and *V. unguiculata* the least magnesium (392 ± 14). Iron concentrations (mg 100 g^{-1} dry weight) were high in *A. thunbergii* (237 ± 8), while

Table 1. Mineral elements in three traditional African leafy vegetables sampled from different geographical regions in South Africa.

Plant species	District	Mineral element concentration (mg 100 g^{-1} dry mass)†				
		Ca	Mg	Fe	Zn	Se
Amaranthus hybridus	Rustenburg	2700.0 ± 70.2	1400.0 ± 0.3	14.8 ± 0.5	0.6 ± 0.2	0.8 ± 0.3
Amaranthus hybridus	Vhembe	1772.2 ± 61.4	871.7 ± 30.2	94.9 ± 3.3	4.2 ± 1.5	0.3 ± 0.01
Amaranthus thunbergii	Capricorn	1932.3 ± 66.9	520.0 ± 18.0	236.8 ± 8.2	12.7 ± 4.4	0.2 ± 0.01
Cleome gynandra	Rustenburg	3100.0 ± 94.7	1311.4 ± 45.4	38.1 ± 1.3	43.7 ± 15.2	0.5 ± 0.01
Cleome gynandra	Capricorn	1943.7 ± 67.3	847.6 ± 29.3	89.7 ± 3.1	8.4 ± 2.9	0.2 ± 0.01
Vigna unguiculata	Vhembe	1722.2 ± 59.7	392.3 ± 13.6	97.9 ± 3.4	6.1 ± 0.1	0.2 ± 0.01

†Mean ± s.d. (n = 3)

in other samples they ranged between 14 ± 0.5 (*A. hybridus*; Rustenburg) and 98 ± 3 (*V. unguiculata*). Iron concentrations in *C. gynandra* were higher in the Capricorn sample (90 ± 3) than in the Rustenburg sample (38 ± 1). Zinc concentrations (mg 100 g^{-1} dry weight) varied between 0.6 ± 0.2 (*A. hybridus*; Rustenburg) and 44 ± 15 (*C. gynandra*; Rustenburg). Comparing these values, zinc was higher in the Vhembe samples of *A. hybridus* (4 ± 2) and *A. thunbergii* (13 ± 4) and lower in the Capricorn sample of *C. gynandra* (8 ± 3). The highest selenium concentrations were in the Rustenburg samples of *A. hybridus* and *C. gynandra* (0.8 ± 0.3 and 0.5 ± 0.01, respectively).

Total phenolics concentration (Table 2)

Total phenolics concentration, expressed as mg GAE 100 g^{-1} dry mass, ranged from 1 057 ± 62 to 2 906 ± 95; the highest value was obtained for *V. unguiculata.* When comparing the amaranth samples, the highest concentration of total phenolics was obtained for *A. hybridus* from Rustenburg (2 181 ± 30), followed by *A. thunbergii* from Capricorn (1 138 ± 42), and the lowest concentration was obtained for *A. hybridus* from Vhembe (1 057 ± 62). *Cleome gynandra* from Rustenburg was also higher in total phenolics (1 924 ± 87) compared with the sample of *C. gynandra* obtained from Capricorn (1 659 ± 30).

Total carotenoids concentration (Table 2)

The highest total carotenoid concentration, expressed as mg 100 g^{-1} dry mass, was measured in *V. unguiculata* (195 ± 5). The total carotenoid concentration of *C. gynandra* was higher in the Rustenburg sample (162 ± 1) compared with the Capricorn sample (94 ± 4). In amaranth samples, the total carotenoids concentration was lowest in *A. thunbergii* (89 ± 11) and highest in the Vhembe sample of *A. hybridus* (131 ± 11).

Beta-carotene concentrations (Table 2)

Beta-carotene concentrations (mg 100 g^{-1} dry mass) ranged from 0.4 ± 0.1 (*C. gynandra;* Rustenburg) to 18.4 ± 1.5 (*A. hybridus;* Rustenburg). The concentrations of beta-carotene were considerably lower in *A. thunbergii* (1.6 ± 0.2) and the Vhembe sample of *A. hybridus* (1.6 ± 1.1) compared with the Rustenburg sample of *A. hybridus* (18.4 ± 1.5). The beta-carotene concentration was notably higher in the Capricorn sample of *C. gynandra* (1.7 ± 0.6) compared with the Rustenburg sample. The concentration of beta-carotenes in *V. unguiculata* measured 3.8 ± 0.3 mg 100 g^{-1}.

Discussion

Toxic radical molecules are continuously generated from plant cellular structures that are involved in photosynthesis and respiration. For protection, plants manufacture organic detoxification molecules in which iron, zinc and selenium feature as essential structural components. Calcium cations (Ca^{2+}) play a vital role in regulating cellular transmembrane trafficking of elements and molecules.[4] Dark-green leafy vegetables, therefore, are primary sources of minerals, trace elements, and antioxidant molecules, such as polyphenols and carotenoids, all of which function in enzymatic and/or non-enzymatic-mediated plant defences against radiation-induced oxidative stress.[17] In the present study, leaves of wild-growing varieties of two amaranth species (*A. hybridus* and *A. thunbergii*), two samples of cat's whiskers (*C. gynandra*) and one of cowpea (*V. unguiculata*) were analysed for their mineral and trace elements, total phenolics, total carotenoids and beta-carotene concentrations. Concentrations of the minerals (calcium and magnesium) and trace elements (iron, zinc and selenium) varied in samples of the same plant species from different geographical localities (Table 1). However, the mineral and trace element content of plant leaves is a function of the environment, and in leafy vegetables would be strongly influenced by the chemical composition of the soil and the climate.[18,19] The highest concentrations of calcium (2 700 mg 100 g^{-1} and 3 100 mg 100 g^{-1}) and magnesium (1 400 mg 100 g^{-1} and 1 311 mg 100 g^{-1}) were measured in *A. hybridus* and *C. gynandra,* respectively, both sampled from the Rustenburg area. These samples also, respectively, contained the lowest and highest zinc concentrations (0.6 mg 100 g^{-1} and 44 mg 100 g^{-1}), suggesting that zinc was differently absorbed by these two plant species. Interestingly, iron concentrations in samples from the North West Province were low (15 mg 100 g^{-1} and 38 mg 100 g^{-1}) in comparison with those samples collected from the Limpopo Province (which ranged from 90 mg 100 g^{-1} in *C. gynandra* to 237 mg 100 g^{-1} in

Table 2. Total phenolics, total carotenoids and beta-carotene in three traditional African green leafy vegetables sampled from different geographical regions in South Africa.

Plant species	District	Total phenolics† (mg GAE 100 g^{-1} dry mass)	Total carotenoids† (mg 100 g^{-1} dry mass)	Beta-carotene† (mg 100 g^{-1} dry mass)
Amaranthus hybridus	Rustenburg	2181.2 ± 30.2	113.6 ± 9.3	18.4 ± 1.5
Amaranthus hybridus	Vhembe	1057.3 ± 61.9	131.3 ± 10.7	1.6 ± 1.1
Amaranthus thunbergii	Capricorn	1137.7 ± 41.9	88.6 ± 10.7	1.6 ± 0.2
Cleome gynandra	Rustenburg	1923.9 ± 87.2	162.3 ± 1.1	0.4 ± 0.1
Cleome gynandra	Capricorn	1659.1 ± 30.0	93.9 ± 3.9	1.7 ± 0.6
Vigna unguiculata	Vhembe	2905.9 ± 94.5	194.9 ± 5.0	3.8 ± 0.3

†Mean ± s.d. (n = 3)

A. thunbergii). Selenium concentrations were low in all the samples. The mineral concentrations measured in the present study are comparable with calcium (2 365 mg 100 g^{-1} and 3 931 mg 100 g^{-1}) and magnesium (1 317 mg 100 g^{-1} and 1 166 mg 100 g^{-1}) concentrations in *A. hybridus* and *A. spinosis,* respectively, that were reported by Odhav *et al.*[20] Both these species that were sampled from assorted habitats (i.e. disturbed land, roadside and field) in KwaZulu-Natal, measured 21 mg 100 g^{-1} of iron and 18 mg 100 g^{-1} of zinc. If, for purposes of comparison, values indicated in the South African Food Composition Data (SAFCOD)[21] are converted to dry mass, commercially-grown spinach and swiss chard, respectively, are lower in both calcium (832 mg 100 g^{-1} and 1 182 mg 100 g^{-1}) and magnesium (664 mg 100 g^{-1} and 788 mg 100 g^{-1}), are comparable in their iron (30 mg 100 g^{-1} and 44 mg 100 g^{-1}) and zinc (4.5 mg 100 g^{-1} and 7.4 mg 100 g^{-1}) concentrations, and higher in selenium (8.4 mg 100 g^{-1} and 12 mg 100 g^{-1}). Results from the present study, and those reported by Odhav *et al.*,[20] suggest wild *morogo* should be considered an important source of calcium, magnesium, iron and zinc, particularly for households that are not in a position to access conventional vegetables, whether for economic or demographic reasons.

Carotenoids, pigment molecules responsible for the colour of many fruits and vegetables, have important functions in photosynthesis and are abundant in plant leaves. Beta-carotenes, for example, are prevalent in photosystem I.[22] Carotenoids have important biological functions and those derived from plant foods can be biologically transformed to provitamin A which is converted to vitamin A only when needed by the body.[23] Faber *et al.*[24] proposed that the majority of children in South Africa between 2 years and 5 years of age consume low nutrient-dense diets which, in addition to other constituents, are also deficient in vitamin A. Another study found that, for children in rural areas, consumption of *morogo* contributed significantly to their intake of calcium and iron, but the biggest nutrient contribution of *morogo* consumption was towards the total intake of vitamin A.[25] Carotenoid and beta-carotene concentrations measured in the three species of wild *morogo* are shown in Table 2. The total carotenoids concentrations in *A. hybridus* (131 mg 100 g^{-1}), *C. gynandra* (162 mg 100 g^{-1}) and *V. unguiculata* (195 mg 100 g^{-1}) compared well with that of baby spinach (140 mg 100 g^{-1}) reported by Bergquist.[22] The beta-carotene contents of the Rustenburg sample of *A. hybridus* (18 mg 100 g^{-1}) and of the unspecified amaranth species reported in the SAFCOD table (16 mg 100 g^{-1})[21] were markedly higher than concentrations measured in *A. thunbergii* (1.6 mg 100 g^{-1}), *C. gynandra* (0.4 mg 100 g^{-1} and 1.7 mg 100 g^{-1}) and *V. unguiculata* (3.8 mg 100 g^{-1}). In commercially-grown *A. tricolor,* levels of total carotenoids (251 mg 100 g^{-1}) and beta-carotene (39 mg 100 g^{-1}) were much higher. Beta-carotene concentrations of 52.9 mg 100 g^{-1} and 291 mg 100 g^{-1} have been reported for *A. gangeticus* and *A. viridis,* respectively.[7,26] According to De Pee and Bloem,[27] the bioavailability of carotenoids in DGLVs is reduced by the leaf matrix. Notwithstanding this limitation, and distinct from being vitamin A precursors, carotenoids also exhibit considerable antioxidant capacity based on their symmetrical linear 40-carbon tetraterpene structure, which features alternating double and single carbon-carbon bonds.[7,16,23]

Polyphenols are another class of phytochemicals that contribute considerably towards the total antioxidant capacity of DGLVs.[28] Grouped together on the basis of their structures having aromatic rings, antioxidant activities of polyphenols are mainly through the donation of hydrogens.[29] Results in Table 2 indicate that wild-growing varieties of *Amaranthus, Cleome* and *Vigna* spp. contained phenolic compounds in amounts comparable to those of conventional and commercially-grown non-conventional vegetables. Total phenolics concentrations were 2 181 mg 100 g^{-1}, 1 924 mg 100 g^{-1} and 2 960 mg 100 g^{-1} in *A. hybridus, C. gynandra* and *V. unguiculata,* respectively, and 2 100 mg 100 g^{-1} in commercial spinach.[30] Lako *et al.*[7] reported total phenolics concentrations of 2 000 mg 100 g^{-1} in leaves of commercially-produced *Ipomaea batata,* which is also eaten as *morogo* in South Africa. Odhav *et al.*[20] demonstrated that methanolic plant extracts (100 mg ml^{-1}), prepared from wild-growing varieties of *A. hybridus, A. spinosus* and *C. monophylla,* exhibited radical scavenging capacities of 90%, 88% and 84%, respectively, relative to the 100% of the positive control, flavonoid rutin.

Polyphenol-rich plant extracts reportedly show protection against atherogenesis by inhibiting oxidation of low density lipoproteins in endothelial cells and macrophages.[6,28] Collins linked the decreased cancer incidence following dietary beta-carotene supplementation to antioxidant protection enhancing resistance to reactive oxygen species (ROS)-induced DNA strand breaks.[31] Other studies ascribed the protective properties of dietary polyphenols and carotenoids against chronic diseases to the ability of these compounds to quench singlet oxygen or scavenge ROS, thus interrupting the transfer of radical reactions from one cell to adjacent cells.[28,29,32] Based on results of phytochemical analysis reported in the present study, and the radical scavenging activities of *morogo* extracts demonstrated by Odhav *et al.*,[20] it seems likely that consumers could derive a range of dietary antioxidants from wild *morogo.*

Wild *morogo* thus appears to be a good source of minerals, trace elements and antioxidants—the consumption of which could be particularly important in resource-poor households who are most likely to suffer deficiencies of these nutrients. Because of their unsatisfactory nutritional status, members of such households are expected to be more vulnerable to infection and chronic diseases.[33] Mineral, trace element and phytochemical profiles of the wild *morogo* varieties reported in the present study support the view of South African authors who consider *morogo* cropping a feasible strategy for resource-poor populations to access a more diverse, nutrient-dense diet.[25,34,35] Moreover, in a joint publication of the United Nations Development Programme and the Food and Agriculture Organization, *Plant Diversity, Sustainable Livelihoods and the HIV/AIDS Crisis,* Gari expressed the view that wild-growing food plants are an affordable and practical source of nutrition to improve the nutritional status of rural HIV-affected households. The author based this view on the fact that wild edible plants represent an inexpensive, labour-responsive means of improving the trace elements quality of poor diets.[36] *Morogo* crops, because they are derived from indigenous African edible plant species that are adapted to local environmental conditions, grow on soils of limited fertility, are drought tolerant and can be harvested in a short period.[9]

Conclusion

The utilisation of wild *morogo* species seems in line with the ecohealth approach advocated by Lebel,[37] namely that biodiversity conservation could have an important role in dietary diversification, improved nutrition and the betterment of human health. Wild *morogo* varieties gathered from the veld are readily accessible and general consumption thereof in rural settings is expected to improve the nutrient density of poor diets. Moreover, encouraging home-garden cropping of *morogo* vegetables seems an appropriate strategy to enrich high-starch diets of resource-poor populations in both rural and urban settings with health-protective minerals, trace elements and antioxidant phytochemicals.

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