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
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). The veld surrounding the rural villages of Phokeng and Zuurplaat (Rustenburg), Nzuzele Valley (Vhembe) and Dikgale (Capricorn) were searched, in the company of women from the community, for *thepe, leroto* and *dinawu* – 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. *leroto* as *Cleome gynandra* L. and *dinawu* 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 achieved. After cooling, 3 ml of water was added and heating was carefully heated in 1 ml of nitric acid (70%) until clarity was achieved. After cooling, 3 ml of water was added and heating was 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). Calibration standards were prepared using external standards prepared from a 1000 ppm single stock solution of the elements. Calibration factors 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 Lakshiminarayana et al.13 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 each sample was determined, as previously described,13 using Folin-Ciocalteu’s procedure.13 Volumes of 200 µl of extract samples was determined, as previously described,13 using Folin-Ciocalteu’s procedure.13 Volumes of 200 µl of extract samples were nitrogen dried. Residues were dissolved in 1 ml of 80% aqueous methanol using an Ultra Turrax mixer for approximately 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 777 nm (Shimadzu 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 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 under 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 (Shimadzu UV-1601 spectrophotometer). Total carotenoid content is described as mean ± standard deviation in mg 100 g–1 dry mass.

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...
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 carotenoid 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 (Ca²⁺) play a vital role in regulating cellular transmembrane trafficking of elements and molecules. 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. 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. Mineral elements in three traditional African leafy vegetables sampled from different geographical regions in South Africa.](image)

<table>
<thead>
<tr>
<th>Plant species</th>
<th>District</th>
<th>Ca (mg 100 g⁻¹ dry mass)</th>
<th>Mg (mg 100 g⁻¹ dry mass)</th>
<th>Fe (mg 100 g⁻¹ dry mass)</th>
<th>Zn (mg 100 g⁻¹ dry mass)</th>
<th>Se (mg 100 g⁻¹ dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. hybridus</td>
<td>Rustenburg</td>
<td>2700.0 ± 70.2</td>
<td>1400.0 ± 0.3</td>
<td>14.8 ± 0.5</td>
<td>0.6 ± 0.2</td>
<td>0.8 ± 0.3</td>
</tr>
<tr>
<td>A. hybridus</td>
<td>Vhembe</td>
<td>1772.2 ± 61.4</td>
<td>871.7 ± 30.2</td>
<td>94.9 ± 3.3</td>
<td>4.2 ± 1.5</td>
<td>0.3 ± 0.01</td>
</tr>
<tr>
<td>A. thunbergii</td>
<td>Capricorn</td>
<td>1932.3 ± 66.9</td>
<td>520.0 ± 18.0</td>
<td>236.8 ± 8.2</td>
<td>12.7 ± 4.4</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>C. gynandra</td>
<td>Rustenburg</td>
<td>3100.0 ± 94.7</td>
<td>1311.4 ± 45.4</td>
<td>38.1 ± 1.3</td>
<td>43.7 ± 15.2</td>
<td>0.5 ± 0.01</td>
</tr>
<tr>
<td>C. gynandra</td>
<td>Capricorn</td>
<td>1943.7 ± 67.3</td>
<td>847.6 ± 29.3</td>
<td>89.7 ± 3.1</td>
<td>8.4 ± 2.9</td>
<td>0.2 ± 0.01</td>
</tr>
<tr>
<td>V. unguiculata</td>
<td>Vhembe</td>
<td>1722.2 ± 59.7</td>
<td>392.3 ± 13.6</td>
<td>97.9 ± 3.4</td>
<td>6.1 ± 0.1</td>
<td>0.2 ± 0.01</td>
</tr>
</tbody>
</table>

†Mean ± s.d. (n = 3)

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

<table>
<thead>
<tr>
<th>Plant species</th>
<th>District</th>
<th>Total phenolics¹ (mg GAE 100 g⁻¹ dry mass)</th>
<th>Total carotenoids¹ (mg 100 g⁻¹ dry mass)</th>
<th>Beta-carotene¹ (mg 100 g⁻¹ dry mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. hybridus</td>
<td>Rustenburg</td>
<td>2181.2 ± 30.2</td>
<td>113.6 ± 9.3</td>
<td>18.4 ± 1.5</td>
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<td>A. hybridus</td>
<td>Vhembe</td>
<td>1057.3 ± 61.9</td>
<td>131.3 ± 10.7</td>
<td>1.6 ± 1.1</td>
</tr>
<tr>
<td>A. thunbergii</td>
<td>Capricorn</td>
<td>1137.7 ± 41.9</td>
<td>88.6 ± 10.7</td>
<td>1.6 ± 0.2</td>
</tr>
<tr>
<td>C. gynandra</td>
<td>Rustenburg</td>
<td>1923.9 ± 87.2</td>
<td>162.3 ± 1.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>C. gynandra</td>
<td>Capricorn</td>
<td>1659.1 ± 30.0</td>
<td>93.9 ± 3.9</td>
<td>1.7 ± 0.6</td>
</tr>
<tr>
<td>V. unguiculata</td>
<td>Vhembe</td>
<td>2905.9 ± 94.5</td>
<td>194.9 ± 5.0</td>
<td>3.8 ± 0.3</td>
</tr>
</tbody>
</table>

¹Mean ± 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 (2.356 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.²⁹ 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.,²⁹ 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 photosystems.¹² 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.²⁹ 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.²³ Carotenoids 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.²¹ 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)²² 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.²⁴ According to De Pe 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.²⁴,²⁵ Polyphenols are another class of phytochemicals that contribute considerably to 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 hydrogen.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.³¹ reported total phenolics concentrations of 2.000 mg 100 g⁻¹ in leaves of commercially-produced Ipomoea 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. spinosis 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.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.³³ 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.²⁷,²⁸,³² Based on results of phytochemical analysis reported in the present study, and the radical scavenging activities of morogo extracts demonstrated by Odhav et al.,²⁹ 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.³⁵ 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.²⁰,²⁴,³⁵ 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.³⁷

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.
The Morogo Research Programme (MRP) gratefully acknowledges the National Research Foundation (NRF) of South Africa for financial support of this study (Grant – FA2004050600064) and Statistical Consultation Services (North-West University) for statistical processing of results. The MRP is most grateful to the participating households in Nzhelele Valley (Vhembe District) and Dikgale (Capricorn District) in the Limpopo Province, and those of Phokeng and Zuurplaat (Rustenburg District) in the North-West Province of South Africa for providing the morogo analysed in this study.

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