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Indigenous edible plants as sources of nutrients and health benefitting components (nutraceuticals)

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Abstract

The project is being undertaken by the CSIR, in collaboration with the Agricultural Research Council (ARC) and Mintek (South Africa's minerals research organisation). The main aim of the project is to focus on the role of indigenous edible plants in improving food security and the health of communities by providing nutrients as well as other health benefitting components (nutraceuticals). The project reported was conducted in two phases. The main objective of the preliminary phase of the project was to identify indigenous edible plants that could be potential sources of micronutrients as well as health benefitting nutraceuticals. The next phase was to undertake studies to determine whether a selected indigenous plant could be a potential source of antioxidants and other nutraceuticals, and thus be used to develop consumer products. *Amaranthus cruentus* (Arusha) was selected as the plant based on propagation methods developed by the ARC.

The amaranth seeds, leaves, stem and roots were analysed at the CSIR for phenolic and carotenoid content, as well as their antioxidant activity. The phenolic content was highest in the seeds and leaves, with an average 17.6 and 16.5 mg Gallic Acid Equivalents (mg GAE)/g, respectively. These plant parts also had the highest antioxidant activity, determined using 2,2-Di-phenyl-1-picryl-hydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid - diammonium salt) (ABTS) radicals. The average DPPH antioxidant activity of the seeds was 30.1 mg Trolox Equivalents (TE)/g, while that for the leaves was slightly lower at 25.1 mg TE/g. The average ABTS antioxidant activity was 84.8 mg TE/g for the seeds and 84.1 mg TE/g for the leaves. The stem and roots had the lowest phenolic content (2.8 and 1.6 GAE/g), DPPH antioxidant activity (3.3 and 2.8 TE/g) and ABTS antioxidant activity (13.1 and 12.7 TE/g). The carotenoid content was highest in amaranth leaves, followed by the seeds, stem and roots. The major carotenoid identified in the leaves was canthaxanthin (47.8 mg/100 g), followed by β-carotene (28.5 mg/100 g) and lutein (20.2 mg/100 g). The seeds contained significantly lower levels of these carotenoids, while the stem and roots contained trace amounts. There were no detectable levels of zeaxanthin and lycopene in the *Amaranthus* species studied. The level of β-carotene (28.5 mg/100 g) in *Amaranthus cruentus* was higher than that in tomatoes (3.7 mg/100 g), which in turn contained lycopene (14.6 mg/100 g), which was absent from amaranth.

From this study it can be concluded that *Amaranthus cruentus* is potentially a good dietary source of the pro-vitamin A carotenoid (β-carotene). Thus consumption of *Amaranthus* spp may contribute towards reducing vitamin A deficiency among South Africans. Amaranth is also a good source of antioxidants (nutraceuticals) and those identified in this study include canthaxanthin (which has also been reported to be an antitumor agent) and lutein, which is reported to slow down the development of age-related eye diseases.

1. General introduction

The contribution of local plant foods to reducing health risks has always been recognized as part of the local knowledge which forms a greater part of the complex cultural system. Research has shown that many edible plants are rich in specific constituents, referred to as phytochemicals, that may have health promoting effects. The major plant-derived chemical groups now recognized as having potential health promoting effects, at least under some circumstances, are the flavonoids, alkaloids, carotenoids, pre- and pro-biotics, phytosterols, tannins, fatty acids, terpenoids, saponins and soluble and insoluble dietary fibres (Basu, Thomas and Acharya, 2007). These phytochemicals have the potential to be incorporated into foods or food supplements as nutraceuticals.

The term nutraceuticals was coined from "nutrition" and "pharmaceutical" in 1989 by Stephen DeFelice, MD, the founder and Chairman of the Foundation for Innovation in Medicine (FIM), in Cranford, New Jersey, United States of America (Kalra, 2003). The definition of nutraceutical can thus be summarized as "any non-toxic food extract supplement that has scientifically proven health benefits for both disease treatment and prevention" (Dillard and German, 2000). It has been generally stated that the health promoting effects of nutraceuticals and other functional foods are likely due to biochemical and cellular interactions, which together promote the overall health of an individual (Dillard and German, 2000).

In the global marketplace nutraceuticals and functional foods have become a multi-billion dollar industry and tremendous growth is projected, with sales for 2010 being estimated to reach between US $167 billion (Basu *et al*., 2007) and US $187 billion (www.StrategyR.com, accessed 1 July 2010). The major global markets are the United States of America, Europe and Japan, which together by 2007, had a combined share estimated at about 86%. Africa is generally not a competitive market for health supplements. According to Basu *et al*. (2007), the increase in the worldwide use of nutraceuticals and functional foods, as reported by various scientific groups may be associated with some of the following:

- **An increase in public health consciousness.**
- **An aging population**: This is mainly related to managing age-related diseases.
- **Escalating health costs**: Consumers are being forced to seek out more cost-effective alternatives to those provided by structured medicine.
- **Recent advances in research and technology**: Advances in food science and technology, food biochemistry and the nutritional sciences (including nutritional genomics) are providing consumers with fresh access to often supplemented produce with recognizable health benefits previously not available.
- **Changes in government regulations and accountability**: Changes in policies and laws governing the distribution and marketing of food are recognizing the current shift in consumer awareness and accountability of government to the people it represents.
- **Expansion of the global market place**: Better communications and transport for marketable goods, coupled with an increased recognition for propriety patented products, is resulting in a more business-friendly environment for expansion of the industry.

In South Africa the overall demand for health products has grown in recent years for three main reasons:

- Increased health awareness and a better understanding of nutrition and preventative self-medication.
- Successful activities of major market players.
- Growth of health and fitness centres.

The dietary supplement categories making an impact on the South African market include garlic, ginseng, gingko biloba, evening primrose oil, Echinacea, St. John's wort and eye health supplements (http://www4.agr.gc.ca/AAFC-AAC, accessed 29 June 2010). In addition, South Africa has also gained the reputation in the world market as the home to new natural remedies, such as Rooibos tea, Aloe products and Hoodia.

The research on indigenous food plants as sources of nutraceuticals is a new field and needs significant financial investment in the crop science programmes, breeding and postharvest and processing technologies to produce end products. Significant opportunities also exist in using emerging technologies for bioprospecting and the development of innovative products.

2. Main objectives

The objective of the CSIR and ARC research teams was to investigate methods of cultivating local edible plants and determine their potential as sources of nutraceuticals. The activities included a review of available information on South African indigenous plants as sources of health benefitting ingredients (nutraceuticals) as well as the determination of the effect of various cultivation methods on the growth and yield of *Amaranthus cruentus* (Arusha). A preliminary antioxidant activity screening as well as carotenoid characterisation was done at the CSIR.

3. The importance of the research

The nutraceutical industry has a potential of providing an opportunity for economic growth for many developing countries endowed with a rich biodiversity and traditional knowledge of the health effects of certain indigenous plant species (Williams, Pehu and Ragasa, 2006). South Africa is considered as a hotspot for biodiversity with more than 22 000 species occurring (Coetzee *et al*., 1999). This is in the order of about 10% of the world's species, yet despite the richness in plant species relatively few of these plants are utilized economically. In addition to its contribution to the nutraceuticals industry, South Africa's biodiversity and indigenous knowledge could potentially be used to identify concepts and products for different markets, such as edible plants as sources of new and natural colourants and flavourants, nutritional/herbal supplements, as well as sweeteners and for the control of hunger.

The cultivation of indigenous plants has been recognized as an area of research with the potential for diversifying South African agriculture and agri-business. The growing nutraceutical market makes it even more lucrative to investigate the potential of identifying and extracting natural food additives and health promoting substances from indigenous edible plants, some of which have been used as vegetables and for other medicinal uses, such as treating and managing various diseases. There are several reasons for the intensive effort to develop selected indigenous food crops and other edible plants. Some of these can be summarized as follows:

- Currently most indigenous food crops are collected in the wild and have poor yield (Jansen van Rensburg *et al*., 2007).
- There is limited information on production practices (these include nutrient and water use efficiency), postharvest handling, preparation and processing, which guarantee good quality and availability throughout the year.
- Promising genotypes have not been identified; therefore there is a lack of good seed sources.
- Nutritional tests, as well as the identification and testing for phytochemicals, have also not been conducted in extensive research.
- The commercialization of edible plants may create income-generating opportunities for rural poor resource farmers, while conserving the biodiversity of vegetables and other edible plants that are indigenous to the country.

Maintaining the competitive edge is important in research; however, a lack of information sharing does create a disadvantage in terms of filling information gaps and developing priorities, which can lead to repetitive research and a waste of valuable resources. The ARC has already been acknowledged as a centre of excellence for research in indigenous vegetable food crops in South Africa and recognizes the potential of these crops in scientific and economic development, thereby contributing tremendously to the value chain of product development.

4. South African indigenous plants as potential sources of nutraceuticals

South Africa's advantage: Biodiversity

As mentioned earlier, South Africa is exceptionally rich in plant diversity with 22 000 species (Coetzee *etal*., 1999). In addition, the region also has great cultural diversity, with many people still using a variety of plants in their daily lives for food, water, shelter, fuel, medicine and other necessities of life (Van Wyk and Gericke, 2000). In a national survey, Jacobs (2004) summarised the use of South African wild food plants, indicating that about 101 plant species are used as food. Of these, the leaves and stems of 65 plant species were cooked and eaten as relishes or pot-herb 'imifino', the roots, tuber and corms of 26 plant species were collected and fruits and nuts of over 100 shrubs and trees were picked and eaten. Despite South Africa's huge biological resources only a few edible crops have been commercialized and cultivated. Even though there is such diversity, millions of people in Southern Africa still do not have enough food to meet their daily requirements and micronutrient deficiency, particularly of vitamin A, still remains a major health challenge, especially in children below 10 years of age (Faber, van Jaarsveld and Laubscher, 2007).

The observations of Jacobs (2004) have recently been confirmed by studies that have shown that the most reported indigenous edible plants consumed in South Africa are the leafy vegetables, which have long been known in rural communities as an essential food that is consumed with carbohydrate staples (Jansen van Rensburg *et al*., 2007). Although these vegetables have high nutritional value and could potentially play an important role in the prevention of malnutrition, unlike other vegetables, indigenous vegetables have never been produced at a commercial scale despite being consumed by millions of South Africans. Indigenous vegetables will possibly face extinction due to many reasons ranging from attitude, policy and climate change, as well as a lack of understanding of indigenous knowledge production systems (Jansen Van Rensburg *et al*., 2007). The ARC is involved in projects to develop cultivation methods for indigenous fruits and vegetables, and these can be extended to the production of edible plants that have high levels of nutraceuticals.

Local success stories and other lessons to be learnt

South Africa, as mentioned previously, has several successes in the production of health supplements that may be termed nutraceutical. The country is well-known for Rooibos tea, from the plant *Aspalathus linearis* (which is rich in antioxidants), *Aloe ferox* (known for its laxative action) and Hoodia (*Hoodia gornodii*) (an appetite suppressant). In recent years, indigenous herbal tea products have made their way into the market, for example Buchu tea (*Agathosma betulina*) and Honey Bush tea (*Cyclopia genistoides*) (Van Wyk, 2008), and there is still scope for several other products.

To further explore its biodiversity, South Africa could also use experiences from other developing countries that are important producers of nutraceuticals, such as China and India. These countries are well-known for their production of traditional functional food products and nutraceuticals. Both these countries have large populations, in particular in the rural, remote and inaccessible areas, which are totally dependent upon herbal remedies and other naturally available bioresources used to treat common ailments and as general and preventative medications. In both India and China, functional foods and nutraceuticals are available and used as part of traditional diets and medicines. There are no strict pharmaceutical regulations and control in the two countries, and most of the products are available to the consumer directly over the counter (Basu *et al*., 2007). The lack of strict regulations has enabled the nutraceutical industry to grow in the host countries, which has further stimulated trade into other countries.

For South Africa to make a mark in the field of nutraceuticals, advances need to be made in terms of preserving our indigenous knowledge and ensuring that it is used for the benefit of the peoples of South Africa, not only to address health problems and malnutrition, but also to create employment through the establishment of industries.

Documentation of South Africa's edible plants with health benefit

A systematic search for South African edible indigenous plants as sources of health-benefitting properties was conducted through literature surveys and CSIR archive material and from direct communication with the ARC. Some of the plants that were indicated as having health benefits, and in particular where leaves are eaten, are summarized in Table 1. These plants were selected because they can be harvested sustainably and some are fast-growing annual plants.

Table 1: A list of some well-known and less known indigenous edible plants with potential use as sources of nutraceuticals

Family/Plant name (scientific and common names)	Description of use	Health/Wellness benefits indicated	Source of information
Leyssera gnaphalodes (besembossie)	Leaves are used to make an aromatic tea	Energy booster, relaxing	Fox and Young, 1982
Sisymbrium thellugii (wild mustard, wild horse radish, usiqwashumbe, isihlalakuhle)	Boiled leaves are reported to sit well in the stomach	Could be a source of enzymes that aid digestion	Fox and Young, 1982
Cleome gynandra L. (palmbossie, lude, lerotho, rotho, murutivi)	Leaves are cooked to reduce the bitter taste and used as relish, and are also dried	The leaves have been used to cure scurvy (high vitamin C content)	Fox and Young, 1982
Hartogia schinoides (ibonsti, mphymsha)	The leaves are chewed as a thirst quencher and to prevent fatigue	Chewing the leaves reduces inclination for food and habitual chewers are said to be very thin	Fox and Young, 1982
Portulaca afra (porkbush, spekboom, igqwanitsha, isicococo)	The raw leaves are chewed and produce an acidic juice	Lactating mothers are reported to chew the leaves to stimulate or increase milk production	Fox and Young, 1982
Lippia spp (several species are found in South Africa and other parts of the world)	The leaves are prepared as a tea and may be used as seasoning for food preparation	Used for the treatment of respiratory disorders; the leaves are also taken as a gastrointestinal remedy throughout South and Central America, tropical Africa and some European countries	Pascual *et al*., (2001), Viljoen *et al*., (2005)
Amaranthus spp (amaranth, imbuya, thepe, vowa)	The young leaves, growth points and whole seedlings are harvested and cooked as relish	Source of antioxidants, hepatoprotective, diuretic	Jansen van Rensburg *et al*. (2007), Faber *et al*., (2007), Mosha *et al*., (1997), Raju *et al*., (2007)

The limited information from literature on some of the specific health benefits of known edible plants presents scope for a comprehensive study that would make a list indigenous food plants available with known and tested functional and nutraceutical properties. Even in those that are described, there is also a need to identify the bioactive components. The study would start with characterising the plants for some of the known major plant-derived chemical groups now recognized as having potential health-promoting effects. The exercise, although costly, would be worthwhile in order to validate some of the beneficial claims that may need to be made. Scientific evidence is increasingly becoming an important requirement in the marketing and promotion of nutraceuticals.

5. Production practices of Amaranthus (ARC)

Several species of amaranth are consumed in South Africa (Jansen Van Rensburg, 2007). Amaranth is rarely cultivated in South Africa because, as with many other African leafy vegetables, people believe the plants will grow naturally. However, there are reports that in the Mpumalanga and Limpopo provinces women harvest and store amaranth seed, which they then sow widely in their fields when they observe a decline in the amaranth population. Of all the indigenous leafy vegetables, amaranth has been identified as part of the group of species that have the potential to be developed as crops. The other species include *Cleome gynandra* (spider flower).

A study was conducted in order to gain an insight into production practices of vegetable amaranth *(Amaranthus cruentus* (Arusha)) under different spacings, transplanting times and harvesting methods. This work was done at the ARC and will be mentioned briefly in this paper.

A year’s data was collected on the agronomic response of vegetable amaranth to plant population density, planting time and harvesting method and preliminary results indicated that yield was increased by planting amaranth during the wet, warm season. The yield potential was higher at early, rather than later, transplanting. The growth period of this summer season vegetable was difficult to extend until winter, as the winter weather exerted unfavourable effects on its growth and development.

Plant population in excess of the optimum plants per hectare minimized the yield per plant, while the overall yield per hectare remained the same.

6. Preliminary screening of Amaranthus for antioxidant activity and determining its carotenoid profile

MATERIALS AND METHODS

Plant material of *Amaranthus cruentus* (Arusha) was harvested at the ARC's Vegetable and Ornamental Plant Institute in Pretoria. The collected material was de-soiled, washed, separated into four segments (i.e. seeds, leaves, stems and roots) and freeze-dried. The dried plant segments were ground to a fine powder and stored at -20^{0}C, until analysis. The plant parts were analysed for total phenolic content, screened for its antioxidant activity and the carotenoid profile was also determined.

Figure 1: Fresh amaranth plant.

Figure 2: Plant segments prior to freeze-drying.

The determination of total phenolics and antioxidant activity

The Folin Ciocalteu method was used for the determination of total phenolics as described by Dykes *et al*. (2005). Extracts were prepared from the freeze-dried material using acidified methanol.

The antioxidant activity was determined using free radicals generated by DPPH and ABTS reagents that were purchased from Sigma Aldrich. The method used is described by Awika *et al*. (2003). The antioxidants for the ABTS assay were extracted using acidified methanol, while those for the DPPH assay were extracted using 70% aqueous acetone.

The analysis of amaranth carotenoids

The carotenoids were extracted using HPLC grade methanol, followed by tetrahydrofuran and then determined by HPLC using a Diode Array Detector. The standards used to identify the carotenoids were zeaxanthin, canthaxanthin, lycopene and lutein (all purchased from Industrial Analytical, South Africa), as well as β-carotene (from Sigma Aldrich). The method used is described by Rodrigues-Amaya and Kimura (2004).

RESULTS AND DISCUSSION

Table 2: The total phenolic content and antioxidant activity of amaranth (*A. cruentus)* plant segments

Plant segment	Total phenolic content[1]	Antioxidant activity (mg TE/g)	
		DPPH[2]	ABTS[2]
Leaves	16.5±0.7	25.1±0.5	84.1±0.5
Seeds	17.6±0.4	30.1±0.5	84.8±0
Stem	2.8±0	3.3±0.1	13.7±0.5
Root	1.6±0	2.8±0.1	12.7±0

1. Total phenolics – mg Gallic Acid Equivalent (GAE)/g dry weight sample.
2. DPPH and ABTS antioxidant activity values – mg trolox equivalent (TE)/g dry weight sample.

The total phenolic content ranged from 1.6 to 17.6 mg GAE/g dry weight sample, with highest values being observed in seeds and leaves and lowest values in stem and roots, respectively (Table 2). The antioxidant activity values were also high in seeds and leaves and lowest in stems and roots. The DPPH antioxidant activity ranged from 2.8 (roots) to 30.1 (seeds) mg Trolox equivalent per g (TE/g). The ABTS antioxidant activity values ranged between 12.7 to 84.8 mg TE/g, for the roots and seeds respectively. In both assays, the antioxidant activities of the seeds and leaves, had the highest values and the stems and roots the lowest values. From the results it was observed that the higher the phenolic content, the higher the antioxidant activity. The correlation between total phenolics and total antioxidant capacities was also observed by Pasko *et al*. (2009) in their work on *Amaranthus spp* and quinoa seeds.

Although the phenolics analysed in the present samples were not identified, generally, some phenolic compounds that have been reported in amaranth include rutin and its aglycone, quercetin (Kalinova and Dadakova, 2009). In another study, the seed of *Amaranthus hypochondriacus*, was found to containing rutin, isoquercetin and nicotiflorin (Barba de la Rosa *et al*., 2009). It is generally accepted that the antioxidant properties of polyphenols contribute to their health-promoting properties. Furthermore, rutin has been identified as having the potential to prevent and treat colorectal carcinogenesis.

Table 3: The carotenoid content of amaranth (*A. cruentus*) plant segments

Plant	Plant segment	Carotenoid content (mg/100 g)				
		Canthaxanthin	β-carotene	Lutein	Lycopene	Zeaxanthin
Amaranthus	Leaves	47.8±1.4	28.5±1.0	20.2±1.2	ND	ND
	Seeds	10.5±0.6	4.2±0.1	4.2±0.1	ND	ND
	Stems	4.1±0.2	1.8±0.1	2.0±0	ND	ND
	Roots	0.3±0	0.1±0	0.2±0	ND	ND

Plant	Plant segment	Carotenoid content (mg/100 g)				
		Canthaxanthin	β-carotene	Lutein	Lycopene	Zeaxanthin
Tomato fruit	Whole fruit	ND	3.7±0.1	0.6±0	14.6±0.7	ND

ND: not detected

The carotenoid content was highest in the *A. cruentus* leaves, followed by the seeds, stems and lowest in the roots (Table 3). The dominant carotenoid in the leaves was identified as canthaxanthin (47.8 mg/100 g), followed by β-carotene (28.4 mg/100 g) and lutein (20.2 mg/100 g). The β-carotene levels reported in this study is within the range reported by Raju *et al*., (2007) for other varieties of amaranth, such as *A. gangeticus* (18.67 mg/100 g), *A. tristis* (16.76 mg/100 g) and *A. viridis* (58.95 mg/100 g). The β-carotene levels found in the present study are higher than the levels found for *A. cruentus* in other studies as reported by Mosha *et al*., (1997), which were 19.12 mg/100 g. However, these authors also report the presence of α-carotene (10.26 mg/100 g), another pro-vitamin A carotenoid. The variation in β-carotene levels within the amaranth is reported to be due to several factors, some of which could be attributed to the variations in the genetic composition of the plants, the environmental conditions under which the plants were grown, harvesting time and the season of the year, agronomic practices applied to the plants prior to harvest and the harvesting and handling practices prior to analysis (Mosha, Pace, Adeyeye, Laswai and Mtebe, 1997).

Raju *et al*. (2007) also report other carotenoids such as violaxanthin (19.15 to 84.06 mg/100 g), lutein (30.30 to 90.43 mg/100 g) and much lower levels of neoxanthin and zeaxanthin (1.15 to 12.63 mg/100 g and 0.23-1.04 mg/100 g, respectively). It should be mentioned that the carotenoid profile is influenced mostly by the method of analysis and detection levels, and sometimes when the HPLC technique alone is used it may be difficult to differentiate carotenoids that occur within the same class, such as the differentiation of one xanthophyll from another or the carotenes from each other. There is thus a need for more advanced techniques, such as use of HPLC in combination with mass spectroscopy, so that the identity of the carotenoids can be confirmed.

These results thus show that *A. cruentus* is an excellent source of the pro-vitamin A carotenoid, β-carotene. The β-carotene is split into vitamin A molecules during absorption from the intestine. The other carotenoids, although they do not posses pro-vitamin A activity, are important as antioxidants, and may contribute to the prevention of disease. The antioxidant role of carotenoids in amaranth is supported by the observation that high antioxidant activity of *A. cruentus* seed flour may not always be correlated to the phenolic content suggesting that non-phenolic compounds might contribute significant free radical scavenging activity (Nsimba, Kikuzaki and Konishi, 2008). Carotenoids may also have important anti-ageing and anticancer properties, such lutein, which protects the tissues of the retina, while canthaxanthin has been demonstrated to inhibit cancer cell proliferation (Palozza *et al*., 1998).

The seeds and leaves, although with similar levels of total phenolics and antioxidant activity, had significantly different level of carotenoids. It can thus be concluded that antioxidant activity of amaranth seed is mainly due to phenolics, while in the leaves, the carotenoids may play a major role in antioxidant activity. In some cases the two components may act together.

CONCLUSION

The *A. cruentus* leaves had the highest total phenolic content, antioxidant activity and carotenoid content. Thus maximum benefit of the plant may be through the consumption of the leaves as a vegetable, as practiced in some sectors of the South African community. There is thus a large nutritional advantage of promoting the cultivation of *Amaranthus* spp, both from the point of view of supplying pro-vitamin A, and also for their nutraceutical benefit.

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8. Endnote

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Contribution by the team

The paper was written by Nomusa Dlamini, who also did some of the literature review with Gerda Botha and Tshidi Moroka. Tshidi Moroka is also co-ordinating the project with DST. The analysis of the amaranth was done by Judy Reddy and Lauraine Mlotshwa, with guidance from Nomusa Dlamini.