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Nutritional analysis of selected wild edible vegetables of the Vhembe region, South Africa

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Abstract. For rural communities, wild edible plants play a paramount role as foods, which improve their nutritional livelihood and combat food insecurity. The prevalence of Vitamin A, Vitamin C, Fe, and Zn from eight wild edible vegetables were studied. The micronutrients have the highest deficiency prevalence in human in the Vhembe region of the Limpopo Province, South Africa whereas trace elements studied in this research play indispensable roles in the maintenance of health and disease states of humans and domestic animals. Plant samples (leaves), free of infections, were obtained from street hawkers located between Levubu and Makhado in the province. Analysis of Fe, Zn, β -carotene and quantification of Vitamin C was achieved by following methods which are explained in the methodology section with slight modifications. *Amaranthus Hybridus* L. contained high amounts of 226 ± 3.06 mg/100g and 15.5 ± 3.52 mg/100g of Fe and Zn respectively. *Solanum retroflexum* was found to have the highest amount of Vitamin A (β -carotene) of 10.91 mg/100g DW, while *Amaranthus thunbergii* contained the highest (69.11mg/100g DW) amount of Vitamin C. There is a chance of blending wild edible vegetables for consumption. The blending of *Solanum retroflexum* and *Amaranthus thunbergii* is therefore recommended as it promotes Vitamin A and Vitamin C contents in the diet of humans.

Keywords. β -carotene, health, Iron, Vitamin A, Vitamin C, wild edible vegetables, Zinc

1. Introduction

It has been estimated that over a billion people rely on wild vegetables to combat food insecurity and improve their nutritional status (Aberoumand, 2011). Hunger and malnutrition threaten millions of people in sub-Saharan Africa and the estimation percentages of inhabitants still experiencing food insecurity stand at approximately 20% (Jansen van Rensburg et al., 2004). In rural poor areas, wild edible plants are imperative dietary supplements providing trace elements, vitamins, and minerals (Lentini and Venza, 2007). The motivation to utilize wild edible leafy vegetables stems from the fact that they are commonly available and affordable to resource limited individuals. The vegetables are good sources of Vitamin A and C (Steyn et al.,

2001), as well as minerals (Almekinders and Boef, 2000), fibers (Schippers, 2002), carbohydrates (Gruben and Denton, 2004), and proteins (Ahmed, 2006). Trace elements play a pivotal role in maintaining certain physiochemical processes that are essential to life (Oteng et al., 2020). Trace elements are inorganic substances required in small amounts, which include Fe, Cu, Mn, Co, F, I and Zn (Oteng et al., 2020). Iron is a mineral element found in every cell of the body (Mamaboleo et al., 2018) and it is considered very crucial because it forms part of blood cells (Abbaspour et al., 2014).

Notably, Zn is important for the normal growth and function of cells mediating innate immunity cells (Roth et al., 2008). These elements are very indispensable in maintaining necessary homeostatic processes for maintaining a good health state of human and domestic animals (Oteng et al., 2020). For example, Fe deficiency is the main cause of anemia in developing countries, and it affects mostly pregnant women and children (Gautum et al., 2008). Zinc deficiency can adversely affect the growth and function of immune cells resulting to an impaired immune system (Roth et al., 2008). The recommended nutrient intakes of zinc should be followed to meet normative storage requirements from diets with different zinc bioavailability (FAO, 1988).

Vitamins from leafy vegetables have protective effects against damage from free radicals (Gupta and Bains, 2006). Vitamin C plays a significant role in disease prevention (Padayatty et al., 2003). Vitamin C has numerous metabolic roles that are greatly dependent on its reducing properties (Sen et al., 2014). Most notably, it is required for the maintenance of healthy skin, gums, and blood vessels. It is also very famous to have many biological functions including collagen formation, absorption of inorganic iron and it is an antioxidant (Amanobo et al., 2011; Nyonje et al., 2014). It is important to make sure that communities are taking the recommended nutrient intakes for Vitamin C by demographic groups (WHO/FAO, 2004). Table 1 indicates the daily requirements of Vitamin C and other water- and fat-soluble vitamins (FAO/WHO, 2004).

In plants, vitamin A occurs in the form of provitamin A carotenoid, such as lutein, β -carotene, violaxanthin, and neoxanthin (Rodriquez-Amaya, 2001). Vitamin A is very crucial as it plays many functions in the human body, for example in the maintenance of normal vision (FOOD AND NUTRITION BOARD, 2001), gene expression, reproduction, embryonic development and growth, as well as proper immune functioning (Grune et al., 2010). It is worth noting that β -carotene intake helps to balance inadequate retinol supply (Elmadfa and Freisling, 2004). The role of wild edible vegetables as sources of Vitamin A is even more relevant given the prevalence of Vitamin A deficiency in developing countries (WHO, 2009). The estimated level of intake for Vitamin A by population demographics have since been established (Beaton et al., 1993; FAO, 1988). Therefore, utilization of these plants can be a step in the right direction in fighting the prevalence of micronutrient deficiency as a function of malnutrition in sub-Saharan Africa.

Table 1: Recommended daily nutrients intake of Vitamin C and other water- and fat-soluble vitamins (Source: FAO/WHO, 2004)

Group	Water-soluble vitamins					
	Vitamin C ^b (mg/day)	Thiamine (mg/day)	Riboflavin (mg/day)	Niacin ^c (mg NE/day)	Vitamin B ₆ (mg/day)	Pantothenate (mg/day)
Infants						
0–6 months	25	0.2	0.3	2 ^d	0.1	1.7
7–12 months	30	0.3	0.4	4	0.3	1.8
Children						
1–3 years	30	0.5	0.5	6	0.5	2.0
4–6 years	30	0.6	0.6	8	0.6	3.0
7–9 years	35	0.9	0.9	12	1.0	4.0
Adolescents						
Females						
10–18 years	40	1.1	1.0	16	1.2	5.0
Males						
10–18 years	40	1.2	1.3	16	1.3	5.0
Adults						
Females						
19–50 years (premenopausal)	45	1.1	1.1	14	1.3	5.0
51–65 years (menopausal)	45	1.1	1.1	14	1.5	5.0
Males						
19–65 years	45	1.2	1.3	16	1.3 (19–50 yrs) 1.7 (50+ yrs)	5.0
Elderly						
Females						
65+ years	45	1.1	1.1	14	1.5	5.0
Males						
65+ years	45	1.2	1.3	16	1.7	5.0
Pregnant women	55	1.4	1.4	18	1.9	6.0
Lactating women	70	1.5	1.6	17	2.0	7.0

^a Recommended nutrient intake (RNI) is the daily intake which meets the nutrient requirements of almost all (97.5%) apparently healthy individuals in an age- and sex-specific population.

^b See Chapter 7 for details.

^c NE = Niacin equivalents.

^d DFE = Dietary folate equivalents; μg of DFE provided = [μg of food folate + ($1.7 \times \mu\text{g}$ of synthetic folic acid)].

^e Vitamin A values are "recommended safe intakes" instead of RNIs. See Chapter 2 for further details.

^f Recommended safe intakes as μg retinol equivalent (RE)/day; conversion factors are as follows:

1 μg retinol = 1 RE

1 μg β -carotene = 0.167 μg RE

1 μg other provitamin A carotenoids = 0.084 μg RE.

Micronutrients are essential for the normal growth of children. Hunger and malnutrition threaten millions of people in sub-Saharan African countries with an estimation of 20% or more of the inhabitants still experiencing food insecurity (Jansen van Rensburg et al., 2004). It has been estimated that 42.4% of young children living in sub-Saharan Africa are Vitamin A deficient (Aguayo and Baker, 2005). There are disturbing reports from South Africa of low intake of micronutrient-rich foods, despite the government's recommendation of increasing the energy density of foods consumed by children (Labadarios, 1999). Results from the North-West Province of South Africa indicated that more than half of the rural people are undernourished, with children and women of child-bearing age being severely affected (Modise, 2010). In the Limpopo Province, also in South Africa, the high occurrence of poverty has a direct bearing on the increase in under-nutritional status in the Province (Moyo et al., 2010). The highest prevalence of Vitamin A deficiency in the Limpopo Province is among children from households in which homemade bread was highly consumed, and where there was a low intake

of vegetables and fruits in general (Vorster et al., 1997). These findings, therefore, drew our attention to investigate the nutritional status of selected wild edible vegetables found in the Vhembe region of the Limpopo Province, focusing mainly on their Fe, Zn, vitamin C, and β -carotene contents.

2. Materials and Methods

2.1 Selection of plant species for nutritional analysis

Various wild edible vegetables were documented through semi-structured interviews. From all the inventoried wild edible plant species, eight vegetables were selected (Faber et al., 2015). First preference was given to plant species which their nutritional status was never checked in the study area. Some of the plant species inventoried during the current study had a high relative frequency of citation, but they were nevertheless not considered due to the reason that they were analyzed in other studies conducted from the Vhembe District Municipality.

Cautiously, for this study, the cut-off percentage of a citation for the selected vegetables was 10. Calculations of the Relative Frequency of citation (RFC) offered the platform of selecting eight wild edible vegetables (Madikizela et al., 2012). These included *Amaranthus hybridus* L., *Amaranthus thunbergii* Moq., *Bidens pilosa* L., *Cucurbita pepo* L. subsp. *pepo*, *Momordica balsamina* L., *Momordica foetida* Schumach, *Solanum retroflexum* Dunal. and *Sonchus asper* (L) Hill.

2.2 Processing of plant samples

Plant samples, free of noticeable infections, were purchased from street hawkers located between Levubu and Makhado in the Limpopo Province. Leaves of the selected vegetables were obtained from the cultivated farmlands located between Levubu and Makhado. Vegetables like *Solanum retroflexum* Dunal. and *Cucurbita pepo* L. subsp. *pepo* are sold in supermarkets. These were immediately transported to the Botany laboratory of the University of Venda, where the edible parts (leaves) were carefully washed with tap water, and then rinsed with distilled water. After rinsing, materials were blotted dry and the remaining moisture on the leaves was evaporated at room temperature (25 ± 2 °C). Samples were then air-dried on the laboratory benches, thereafter they were ground to a fine powder and stored at room temperature in brown envelopes for the nutritional analysis.

2.3 Nutritional analysis

2.3.1 Determination of β - carotene

Analysis of β - carotene content from the eight selected vegetables was performed, following the method applied by Moyo et al. (2018). Following sample extraction with ice-cold hexane: acetone (1:1, v/v) and centrifugation for 2 min at 2,000 rpm, the separated organic phase filtered through a 0.45 μ m syringe filter was injected (20 μ l) into a Prominence-*i* HPLC-PDA model system equipped with sample cooler LC-2030C (Shimadzu, Kyoto, Japan) (Moyo et al., 2010). A C₁₈ Luna® column (150 x 4.6 mm, 5 μ), which was maintained at 35 °C was used for chromatographic separation with acetonitrile: dichloromethane: methanol (7:2:1) isocratic mobile phase at 1 ml/min flow rate. Detection was set at 450 nm wavelength. An authentic β -carotene standard obtained from Merck® (South Africa) was used for the calibration curve, as well as the identification and quantification of the β -carotene peak. Each determination was done in triplicates.

2.3.2 Quantification of Vitamin C

Quantification of Vitamin C was achieved by following the method described in two previous studies (WHO, 2009; Beaton et al., 1993) with slight modifications (Grune et al., 2010). During the quantification, individual samples of 1g each were weighed into a tube in which 10 ml of 5% metaphosphoric acid was added. The tube was sonicated in an ice-cold water bath for 15 minutes before being centrifuged and filtered. The analysis was carried on a Prominence-i HPLC-PDA model system equipped with cooler LC-2030C. Chromatographic separation achieved using a C18 Luna ® column (150 x 4.6 mm, 5 µ) maintained at 35°C. An isocratic mobile phase made up of water: acetonitrile: formic acid (99:0.9:0.1) at a flow rate of 1ml/min was used. The injection volume of 20 µl was used at the detection set-up of 245 nm. Sample quantification was attained basing on the calibration curve plotted from L-ascorbic acid (Ang and Lee, 2005).

2.3.3 Fe and Zn determination

Iron and zinc contents of the selected vegetables were determined following the method detailed by Ang and Lee (2005) with modifications. Each sample (0.5 g), digested with nitric acid: hydrochloric acid (1:3 v/v; 10 ml) was made to boil on a hotplate at 95 °C until the sample was dissolved. The quantification was done using inductively coupled plasma atomic emission spectroscopy (ICPE-9820, Shimadzu Corporation, Kyoto, Japan). Each determination was done in triplicates.

2.4 Statistical analysis

Data were subjected to one-way analysis of variance using SPSS software (version 16). Where significance was established ($p = 0.05$), the mean values were separated using Duncan's multiple range test.

3. Results

The eight vegetable species contained Vitamin C ranging from 10.11 mg/100 g dry weight (DW) to 69.11 mg/100 g DW (Table 2). Of note is the high Vitamin C concentrations found in the leaves of *A. thunbergii* Moq. and *A. hybridus* L. with values of 69.106 mg/100 g and 43.299 mg/100 g, respectively. Mamaboleo et al. (2010) reported high Vitamin C contents of *Amaranthus cruentus* (Madiira Ex Zim) and *Amaranthus cruentus* (Madiira AM 38) at different stage of maturation. Therefore, it is evident that the high Vitamin C of *Amaranthus* species, studied during the current study, concur with the results of other studies. Furthermore, Akubugwo et al. (2007) reported high value of Vitamin C from *Amaranthus hybridus* L. Ascorbic acid is necessary for healthy teeth, gums and bones and is essential for proper functioning of adrenal and thyroid glands (Akubugwo et al., 2007).

Table 2: Micronutrient analysis of eight selected wild edible vegetables from Vhembe region in South Africa

Species name	B-carotene (mg/100g DW)*	Vitamin C (mg/100g DW)*	Fe (mg/100g DW)*	Zn (mg/100g DW)*
<i>Momordica foetida</i> Schumacher	7.34 ± 0.17 c	10.11 ± 0.05 h	76.00 ± 0.61 cde	ND
<i>Momordica balsamina</i> L.	3.44 ± 0.01 e	20.08 ± 0.09 f	94.73 ± 0.64 cd	5.05 ± 0.27 b
<i>Bidens pilosa</i> L.	2.99 ± 0.09 f	34.94 ± 0.03 c	148.73 ± 70.63 bc	5.41 ± 0.23 b

<i>Solanum retroflexum</i> Dunal.	10.91 ± 0.16 a	30.63 ± 0.18 d	ND	ND
<i>Cucurbita pepo</i> L. subsp. <i>Pepo</i>	6.96 ± 0.03 d	21.90 ± 0.19 e	180.60 ± 0.64 ab	5.87 ± 0.70 b
<i>Amaranthus hybridus</i> L..	10.62 ± 0.03 ab	43.30 ± 0.23 b	229.33 ± 1.76 a	18.98 ± 1.81 a
<i>Amaranthus thunbergii</i> Moq.	7.26 ± 0.21 cd	69.11 ± 0.20 a	ND	ND
<i>Sonchus asper</i> (L.) Hill	10.37 ± 0.01 b	10.89 ± 0.11 g	38.87 ± 0.44 de	1.35 ± 0.71 c

Mean values in each column with different letters indicate a statistically significant difference ($p = 0.05$) based on Duncan's multiple range test done using SPSS software (version 16). ND = Not detected

Of the eight evaluated vegetables, leaves of *M. foetida* were found to have the lowest amount of Vitamin C (Table 1). Low concentrations of Vitamin C contents of six of eight evaluated samples are probably because these samples were dried at room temperature, which might have had a greater influence on them than the two species of *Amaranthus*.

The leaf β -carotene content of the sampled species ranged from 2.994 mg/100 g to 10.905 mg/100 g (Table 1). Most (i.e., six out of eight) of the wild edible vegetables evaluated contain substantial quantities of β -carotene. An exceptionally high value of 10.905 mg/100g β -carotene was from *Solanum retroflexum*. Iron and zinc contents ranged from 0 - 226 mg/100 g DW and 0 - 15.5 mg/100 g DW, respectively (Table 1). *Amaranthus hybridus* L. showed to be a potentially important source of both Fe and Zn with 226 mg/100 g and 15.5 mg/100 g, respectively.

4. Discussion and conclusion

The vitamin C and β -carotene values are higher than those obtained by Nesamvuni et al. (2001), 22.3 mg also for *Amaranthus*. The variance might be due to climatic and soil differences. Nesamvuni et al. (2001) furthermore, indicated higher levels of Vitamin C in species of the family Amaranthaceae compared to Vitamin C contents of plant species of other families. The availability of different vitamins in wild edible plants is species-dependent (Brindisi et al., 2020). This was also evidenced during the current study as some plant species were found to have high levels of vitamin C, whereas some had low levels of vitamin C (Mokganya, 2020). The Food and Agriculture Organization (FAO, 2001), also stated that low Vitamin C concentration in plants can be due to postharvest drying methodology and temperature of storage, which can result in a great loss. Negi and Roy (2001) indicated that storage of dehydrated leafy vegetables should ideally be at low temperatures to reduce the degradation of Vitamin C and browning. All the eight selected wild edible vegetables are first cooked for human consumption; however, *Amaranthus hybridus* L., *Amaranthus thunbergii* Moq., *Momordica foetida* Schumach and *Momordica balsamina* L. are sun-dried and stored in low temperatures later for utilization as spice (Nesamvuni, 2001).

On the other hand, Nesamvuni et al. (2001) also found *S. retroflexum* to have a higher value compared to other investigated species of that study. It must be noted that the levels of Beta-carotene presented here are of raw leaves. Thus, there is the hope of experiencing some increments of these levels if dried leaves are used as spices instead of cooking before consumed by humans.

Nutritional analyses of the eight selected wild edible vegetables revealed that some of these plant species are good sources of β -carotene and Vitamin C. *Amaranthus thunbergii* and *Amaranthus hybridus* showed high levels of Vitamin C than *Solanum retroflexum* and *Sonchus asper* which had high levels of β -carotene. There are possibilities of combining the above-

mentioned vegetables for human consumption. In that case, simultaneous consumption of these vegetables can amplify their potential in the fight against micronutrient deficiency. Consumption of these indigenous vegetables is therefore highly recommended as it can help address challenges of food insecurity as well as addressing hidden hunger. A further recommendation is with promotive campaigns of these underutilized vegetables in combating malnutrition.

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