

Health Promoting Potentials of Selected South African Indigenous Leafy Vegetables

George G¹⁺, Ganjifrockwalla F¹ and Gqaza BM¹

¹ Division of Medical Biochemistry, Department of Human Biology, Faculty of Health Sciences, Walter Sisulu University, Mthatha, South Africa

Abstract. Six dietary leafy vegetables commonly consumed by the rural Eastern Cape communities of South Africa were analysed for their antioxidant activity and phenolic content. The indigenous leafy vegetables (ILVs) were *Amaranthus hybridus*, *Solanum nigrum*, *Bidens pilosa*, *Urtica urens*, *Ribes uva crispera* and *Taraxacum officianale*. Aqueous and methanol extracts were tested for free radical scavenging and antioxidant activity using 2,2-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) assay and expressed as trolox equivalents. Total phenolic content was determined using the Folin-Ciocalteu assay with gallic acid as the standard and expressed as gallic acid equivalents. The antioxidant activity of the indigenous leafy vegetables for aqueous extracts ranged from 1.28 – 11.45 mmol/100g and the range observed for methanol extracts were 7.85 – 17.2 mmol/100g. The total phenolic content of the water extracts were 226.7 – 651.7mg/100g. The phenol content in the methanol extracts were in the range of 187.8 – 764.9 mg/100g gallic acid equivalent (GAE)/100g. Total antioxidant activity correlated well with total phenolic content of the methanol extract ($R^2 = 0.875$). Results suggest that the indigenous leafy vegetables tested in the study have the potential to provide antioxidant properties and may offer effective protection from free radical injury implicated in metabolic stress, chronic and degenerative diseases if included in the diet.

Keywords: indigenous leafy vegetables, antioxidant activity, total phenolic content, metabolic stress, chronic and degenerative diseases.

1. Introduction

Many of the leafy vegetables grown spontaneously in natural ecosystems have been serving human kind as food and medicine in almost all societies. [1] The tradition of eating wild leafy vegetables have declined in urbanized societies due to their non-availability in urban markets and as the societies got used to the cultivated domesticated varieties. [2]. Wild leafy vegetables are considered to be famine foods by modern societies but still accepted as appropriate and desirable by many rural communities around the world particularly in Sub Saharan Africa. Indigenous leafy vegetables gathered from the field and around the homestead formed an important part of the rural diets for centuries in most of the developing nations including South Africa. The agro-biodiversity of South Africa is rich and consists of a wide variety of indigenous leafy vegetables popularly referred to as imifino (isi Zulu/isiXhosa), morogo (Sesotho/isiPedi) and muhoro (Tshivenda) in different language speaking areas. [3] Many different authors have documented the use of indigenous leafy vegetables in South Africa.

[3]-[5] Very few have been studied for their macro micro nutrient content. [2], [3], [6], [7] Some of the leafy vegetables have also been studied for their bioactive phytochemical particularly antioxidant contents [8], [9].

Recent scientific evidence suggest that oxidative stress which result in the generation of free radicals contribute to oxidative damage of tissue. Free radicals generated by cellular processes are generally

⁺ Corresponding author. Tel: +27835701221; fax: +27475022425
E-mail address: ggeorge.grace@gmail.com

scavenged by the endogenous and exogenous (dietary) antioxidants present in the system. When there is an imbalance between the oxidant and antioxidant systems damage of structural and functional components of the cell due to oxidative stress occur and has been implicated as primary or secondary causative factors in many different human diseases including cancer, hypertension, cardiovascular diseases, secondary complications of diabetes and many others. [10] Epidemiological studies have shown protective effect of dietary antioxidants against the risk of free radical induced injury to the body. A high intake of food rich in natural antioxidants has been shown to increase the antioxidant capacity of plasma and reduce the risk of development many chronic and degenerative diseases.[11], [12] Recent research also has shown that through complimentary action and effects of a complex mixture of phytochemical compounds in fruits and vegetables provide a protective effect on health than a single isolated phytochemical, meaning that a whole fruit or vegetable is better than a product made from them. The protective effect is attributed to many components including vitamin C, E, carotenoid pigments, phenolic compounds and other phytochemicals present in fruits and vegetables. [13]

Based on the above background the present work was undertaken to study the invitro antioxidant potentials and their corresponding polyphenol content of six indigenous leafy vegetables commonly consumed by the rural population in the Eastern Cape region of South Africa.

2. Materials and Methods

The plant species investigated in the present study include the following:

<p><i>Amaranthus hybridus</i> (Smoothpigweed/ unomdlomboyi)</p> 	<p><i>Bidens pilosa</i> (Black jack/ umhlabangulo)</p> 
<p><i>Solanum nigrum</i> (Blacknightshade/ Umsobo)</p> 	<p><i>Urtica urens</i> (Dwarf nettle Ububazi)</p> 
	
<p><i>Ribes uva crisa</i> (Iguzu)</p>	<p><i>Peddiae Africana</i> (ugcamche)</p>

The above plant materials were collected, and identified and authenticated in the Botany laboratory by Dr Immelman and voucher specimens were deposited in the Walter Sisulu University herbarium.

2.1. Processing of Plant Material and Extract Preparation

The plant materials were dried in the shade and pulverised using laboratory grinder and stored in air tight glass containers. Three grams (3 g) of each plant material were extracted with water (aqueous extract) and 70% aqueous methanol (Methanol extract) by sonication for 30 min and centrifuged for 5 min at 4000 rpm. The extracts were filtered using Whatman no. 1 filter paper. Both aqueous and methanol extracts were used for assays

Aliquots were analyzed for their antioxidant capacity using **ABTS radical scavenging assay** and total phenol content using **Folin–Ciocalteu assay** methods. Each sample was prepared and analyzed for each assay in triplicate. All reported values for each sample and in the calculation of correlation coefficient (R^2), the mean values of three estimations were considered.

2.2. ABTS Radical Scavenging Assay

The free radical scavenging activity of the plant extracts were determined using 2,2' azinobis- (3-ethylbenzthiazoline-6-sulphonic acid) (ABTS) radical cation decolourization assay. 6-hydroxy 2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox) was used as a standard according to Re et al 1999. [14] The antioxidant capacity based on the ABTS free radical scavenging ability of the extract was expressed as mmol Trolox equivalent antioxidant capacity (TEAC) per 100 g of plant material.

2.3. Folin- Ciocalteu Assay

Total phenolic concentration in plant extracts were determined spectrophotometrically at 760nm by Folin-Ciocalteu assay as described by Singleton and Rossi, (1965) using gallic acid as the standard. [15] The total phenolic content of the plant extracts were expressed as mg gallic acid equivalents (GAE) per 100g of plant material

3. Results and Discussion

The results (Table 1) indicated that all indigenous leafy vegetables analysed exhibited antioxidant activity for aqueous and methanolic extracts. Antioxidant activity expressed as trolox equivalent capacity (TEAC) ranged from 1.28- 11.45 mmol/100g for aqueous extract.

Table 1: Total antioxidant activity and Polyphenol content of plant extracts

Plant species	ABTS	ABTS	Polyphenol	Polyphenol
	Aqueous Extract TEAC mmol/100g	Methanol Extract TEAC mmol/100g	Aqueous Extract mg GAE/100g	Methanol Extract mg GAE/100g
<i>Amaranthus hybridus</i>	4.71	9.04	329.0	309.5
<i>Solanum nigrum</i>	5.81	14.30	651.7	722.33
<i>Bidens pilosa</i> ^a	11.45	17.21	548.4	921.7
<i>Bidens pilosa</i> ^b	4.19	14.87	226.7	731.7
<i>Peddiea africana</i>	4.06	7.85	380.66	187.88
<i>Urtica urens</i>	4.33	10.2	379.55	286.22
<i>Ribes uva crispa</i>	1.28	12.5	490.66	764.00

^a Plant collected from Mthatha, Eastern Cape, South Africa ^b Plant collected from Bizana, Eastern Cape, South Africa

ABTS - 2,2 azinobis-3-ethylbenzthiazoline-6-sulphonic acid assay

TEAC - Trolox equivalent, GAE – Gallic acid equivalent

Ribes uva crispa (Iguzu) showed the lowest values while *Bidens pilosa* (black jack) was the highest. TEAC for methanol extracts of the leaves varied from 7.85 – 17.24mmol/100g. *Peddie Africana* methanol extracts recorded the lowest activity while *Bidens pilosa* extracts were the highest. It was also noteworthy that *Bidens pilosa* collected from two different locations in the same geographical area varied in their antioxidant capacity for both aqueous (4.19& 11.45mmol/100g) and methanol extracts (14.87 & 17.21mmol/100g).

The polyphenol content of the aqueous extracts of indigenous leafy vegetables studied varied from 226.7 – 651.7mg GAE/100g while the methanolic extracts recorded 286.2- 921.7 mg GAE/100g plant material.

Between two *Bidens pilosa* samples there was difference in the polyphenol content and antioxidant activity observed in the TEAC values. Higher the polyphenol content higher was the antioxidant activity. ABTS method has been reported to be suitable for both hydrophilic and lipophilic antioxidants as ABTS is soluble in both aqueous and organic solvents. [16] *Bidens pilosa* samples collected from Mthatha region was richer in both antioxidant activity and polyphenol content. The observed differences may be due to the climatic and stress conditions prevalent in the respective areas as bioactive phytochemicals are the secondary metabolites of the plants which are synthesized in response to stress in plants.

The results of Pearson correlation analysis of antioxidant activity and polyphenol content of the extracts showed a significant correlation ($P < 0.01$) between the antioxidant activity of the methanolic extracts and polyphenol content ($R^2 = 0.875$).

Vegetables and fruits are generally rich sources of macro and micro nutrients as well as health promoting phytochemicals. Phytochemicals which appear to have health promoting potential are tetraterpenoid pigments carotenoids, phenolic compounds (flavonoids, phytoestrogens and phenolic acids), phytosterols, phytosterols, tocotrienols, organosulfur compounds, and non-digestible dietary fiber. [12] Several investigators have reported antioxidant activities of many different green leafy vegetables and these activities are attributed to their polyphenolic compounds [8], [9], [11]. The observation of positive correlation of antioxidant activity and polyphenol content in methanol extracts may be expected because of the enhanced extraction of compounds in the methanol. Similar observations were reported earlier by other authors [8]. Generation of free radicals and reactive oxygen species occur during the normal metabolism of the body and are removed by the endogenous antioxidant systems in the body. When the production of these compounds exceed the capacity of the living systems it is manifested as disease. Many polyphenols derived from diet act as scavengers of free radicals, quenchers of singlet and triplet oxygen and inhibitors of peroxidation in the living systems and protect the integrity of structural and functional components. [17], [18] It may also be noted that some of the plants used in this study has been reported in literature as having antimicrobial (*Urtica urens*) [9], antidiabetic (*Bidens pilosa*) [19], and chemopreventive (*Solanum nigrum*). [20]

4. Conclusion

All six plants used in the study showed good antioxidant potential in in-vitro experiments. The antioxidant effects shown by aqueous extracts of these plants are encouraging since people consume these vegetables after cooking. The great biodiversity of South African ILVs are unstudied or understudied for their bioactive compounds and health promoting potential. The results of this study is expected to fill in some gaps in the data base and may assist in the evidence based utilization of these ILVs. Further studies are required to assess the in-vivo effect of these plants because beneficial effect in providing antioxidant protection depend on the bioavailability of polyphenols in these plants. If the polyphenols are bioavailable from these leaves they could serve as health promoting functional foods which are cheaper for the rural population.

5. Acknowledgement

The authors wish to express their gratitude to Walter Sisulu University Research Development office for the financial support for the work.

6. References

- [1] Food and Agricultural Organization. Use and potential of wild plants in farm households. Farm systems management series version 15, 1999
- [2] B Odhav, S Beekrum, US Akula, H Baijmath. Preliminary assessment of nutritional leafy vegetables in KwaZulu Natal South Africa. *J. Food Comp. Anal.* 2006, **20** (5): 430-435
- [3] WS Jansen van Rensburg1, W van Averbeke, R Slabbert, M Faber, P van Jaarsveld, I van Heerden, F Wenhold and A Oelofse. *Water SA. (Special Edition)* 2007, **33**(3): 317 – 326
- [4] TGB Hart and HJ Vorster. Indigenous Knowledge on the South African Landscape – Potentials for Agricultural Development. Urban, Rural and Economic Development Programme. 2006, Occasional paper No 1. HSRC Press,

Cape Town, South Africa. 52 pp.

- [5] RB Bhat and T Rubuluza. The bio-diversity of traditional vegetables of the Transkei region in the Eastern Cape of South Africa. *South African Journal of Botany* 2002, **68** (1): 94-99
- [6] C Nesamvuni, NP Steyn and MJ Potgieter. Nutritional value of wild, leafy vegetables consumed by the VhaVhenda. *South African Journal of Science*, 2000, **97**: 51-54.
- [7] NP Steyn, J Olivier , P Winter , S Burger and C Nesamvuni. A survey of wild, green leafy vegetables and their potential in combating micronutrient deficiencies in rural populations. *South African Journal of Science*. 2001, **97**(7/8): 276-278
- [8] D Katerere, G Graziani, KM Thembo, NZ Nyazema and A Ritieni. Antioxidant activity of some African medicinal dietary leafy vegetables. *African Journal of Biotechnology*. 2012, **11**(17):4103 – 4105
- [9] F Jimoh, A Adedapo, A Aliero and A Afolayan. Polyphenolic and biological activities of leaves extracts of *Argemone subfusiformis* (Papaveraceae) and *Urtica urens* (Urticaceae), *International Journal of Tropical Biology*. 2010, **58** (4): 1517 – 1531
- [10] B D Cox, M J Whichelow, and A. T Prevost, “Seasonal consumption of salad vegetables and fresh fruits in relation to the development of cardiovascular disease and cancer,” *Public Health Nutrition*. 2000, **3** (1) 19–29
- [11] M A Eastwood, Interaction of dietary antioxidants *in vivo*: how fruit and vegetables prevent disease?”. *QJM*, 1999, **92**(9) 527–530.
- [12] EB Rodriguez, ME Flavier, DB Rodriguez- Amaya, J Amaya- Farfan. Phytochemicals and functional foods, Current situation and prospects for developing countries. *Seguranca Alimentar, Nutricional,Campinas*. 2006, **13** (1) 1-22
- [13] N Cotelle. Role of flavonoids in oxidative stress. *Current Tropical Medical Chemistry*, 2001, **1**(16) 569 -590
- [14] R Re, N Pellegrini, A Proteggente, A Pannala, M Yang and C Rice-Evans. Antioxidant activity applying an improved ABTS radical scavenging cation decolorization assay. *Free radical Biology and Medicine*. 1999, **26** (9/10) 1231-1237
- [15] VL Singleton & JA Rossi. Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. *American journal of Enology and Viticulture*. 1965 **16**(3), 144-158.
- [16] MB Arnao. Some methodological problems in the determination of antioxidant activity using chromogen radicals: A practical case. *Trends Food Science and Technology*. 2000, **11**, 419 – 421
- [17] CA Rice Evans, NJ Miller, G Paganga. Structure- activity relationships of flavonoids and phenolic acids. *Free Radical Biology and Medicine*. 1996,**20**: 933-56
- [18] RI Prior, G Cao. Antioxidant phytochemicals in fruits and vegetables. *Horticultural Science*. 2000,**35**: 588-92
- [19] Shih-Chang Chien a,1, Paul H. Young a,1, Yi-Jou Hsu a,1, Chun-Houh Chen b, Yin-Jing Tien b,c, Shang-Ying Shiu b, Anti-diabetic properties of three common *Bidens pilosa* variants in Taiwan. *Phytochemistry* 2009, **70**: 1246–1254
- [20] Ahmed M. ABOUL-ENEIN*1), Faten Abu EL-ELA1), Emad Shalaby and Hany EL Shemy, Potent Anticancer and Antioxidant Activities of Active Ingredients Separated from *Solanum nigrum* and *Cassia italica* extracts. *Journal of Arid Land Studies* 2014, **24**(1): 145 – 152.