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Phytochemical and Antioxidant Composition of Selected Local Wild Plants in South Africa: Consideration of Alternative Nutrients for Health Promotion

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Abstract. Plant foods used as vegetables are recommended constituents of the daily diet as they are essential sources of nourishment and if used as a complementary vegetable to the starch and protein staple foods they become a more vital health ingredient to balance the diet with micronutrients.
Procedures were used to determine the total phenols, flavonoids, flavonols and proanthocyanidins from the fresh leaves of *Chenopodium album* (fat hen/goosefoot), *Solanum nigrum* (black nightshade), *Urtica lobulata* (stinging nettle) and *Amaranthus dubius* (wild spinach). The antioxidant activity was screened through the DPPH, ABTS and FRAP radical scavenging effects. Values of the total flavonoid content in the four selected species ranged from 9.14 in C.*album* to 13.30 mg/g dry weight in S. *nigrum*, whereas those of total phenolics were generally much higher and varied between 9.34 in *C. album* to 30.00 mg/g dry weight in *S. nigrum*. All the plant species had a remarkably high content of proanthocyanidins (between 58.42 and 65.18 mg/g) and also exhibited high radical scavenging activity *in vitro*. Phytochemical compounds contribute to the nutritive value of foods and beverages.
This study has shown that *Chenopodium album, Solanum nigram, Amaranthus dubius and Urtica lobulata* could contribute to the nutrition security of the communities in rural areas in South Africa, as the species are common to most rural areas in the country.

Keywords: Phytochemicals, antioxidants, wild plant foods, phytonutrients, health promotion

1. Introduction

South Africa is a country with abundance of both natural vegetation and exotic food, however, hunger and malnutrition are still found in many rural and peri-urban areas [1]. It has been estimated that about 3 million of people under the age of 15 are suffering from malnutrition as food crisis deepens in South Africa, part of the causes of this are due to low micro-nutrient intake [1]. There is also a burden of more than 45% of the South African adult population suffering from chronic non-communicable diseases such as type II diabetes, cancer, and cardiovascular disease that lead to strokes [1], [2]. Globally, these have been reported to be among the leading causes of death especially in developed nations and they are due to the lifestyle changes amongst people particularly the transition in dietary habits [3]. Conversely, Liu [4] reported on the investigation of phytochemical components of fruits and vegetables being beneficial in preventing a number of these chronic diseases. The scientific enquiry into the phytochemicals by Papp *et al.* (2007) [5] revealed that there may be just above 9 500 different phytochemicals with potential to prevent these chronic diseases including others such as metabolic syndrome, mentally associated-Alzheimer's disease, eye-related diseases-cataracts and age-related functional decline.

The term phytochemical generally, is used to refer to chemical compounds that occur naturally in plants which are responsible for color and organoleptic properties, such as the deep purple of blueberries and the

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typical smell of onion, garlic and fruits [6]. Those chemicals have been reported to possess properties of biological significance. Owing to the presence of phytochemicals, many of the wild edible plants have been reported to hold antimicrobial properties, cholesterol lowering effects, anti-diabetic and anti-inflamatory properties [7], [8]. The consumption of plant foods rich in phytochemical nutrients has been documented as important dietary sources with potential to reduce arterial plaque, possess health-protective antioxidant properties and have immune-strengthening properties that can affect these degenerative diseases [9]. Coupled with phytochemical analysis, the search for natural antioxidants sources is gaining much importance. The scientific reports and experimental studies have shown that plants contain a large variety of phytochemicals that have antioxidant properties [10]. The most common plant phenolic antioxidants include flavonoid compounds, cinnamic acid derivatives, coumarins, tocopherols and poly functional organic acids [11]. Much research into free radicals has confirmed that foods or plants rich in antioxidants play an essential role in the prevention of such free radical related diseases [12], [13].

Currently, studies and availability of information on the phytochemical and antioxidant properties of many wild (edible) leafy vegetables in the Eastern Cape Province of South Africa are sporadic and lacking. Studies on antioxidants have been on various other wild medicinal plants and plant products and very few on wild food plants. In this survey four common plants were selected based on their use as edible vegetables in the region in order to analyse the phytochemical content and antioxidant activities of the plants to fill-in the information gap.

2. Materials and Methods

2.1. Plant Collection and Preparation of Extract

Fresh leaves of the dark green vegetables of the *Chenopodium album* L., *(*goosefoot), *Solanum nigrum* L., (black nightshade) *Urtica lobulata* E.Mey. ex Blume, (stinging nettle) *and A. dubius* were collected from various sites of Amathole District in South Africa. Voucher specimens were deposited at the Grahamstown Albany Museum Herbarium for botanical identification and authentication at the Grahamstown Selmar Schonland Herbarium. The collected fresh sample leaves were prepared by drying and grinding into a powder, refrigerated at 40C in airtight labeled volumetric flasks until they were used to determine the phytochemicals. Chemicals used as solvents and reagents were of analytical grade including folin-ciocaltteu's phenol reagent, sodium carbonate, AlCl3, ethanol solution, sodium acetate4% vanillin-methanol, hydrochloric acid, etc.

2.2. Phytochemical Analysis

The concentrated methanol extract of the samples were freeze dried then refrigerated at 4℃. Procedures based on Adedapo *et al.* (2008) [14], were used to determine the total phenols, flavonoids, flavonols and proanthocyanidins. The remainder of the dried extracts obtained was used for the determination of antioxidant activities. The phytochemical contents of four plant samples were quantitatively and qualitatively determined according to modified Adedapo methods. Methods were altered to suit the university resources and the best alternative substitutions were done when a suitable reagent could not be found from the main supplier.

2.3. Determination of Antioxidant Activity

The antioxidant activity of plant extracts were determined by different methods. The free radical scavenging activities of the plant extracts were followed via their reaction with the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method and their ferric ions reducing abilities were determined using the ferric ion reducing antioxidant potential (FRAP) assay [14]. The free radical scavenging activities of the plant extracts were also analysed by the ABTS (2,2'-azino-bis-3-ethylbenzothiazoline-6-sulphonic acid) assay as recommended by Adedapo *et al*., (2008) [14].

2.3.1. DPPH radical scavenging assay

The effect of extracts on DPPH radical was estimated using the method of Liyana-Pathirana and Shahidi (2005) [15]. The reaction mixture was vortexed thoroughly and left in the dark at room temperature for 30

min. The absorbance of the mixture was measured spectro-photometrically at 517 nm. Ascorbic acid and BHT were used as references.

2.3.2. ABTS radical scavenging assay

Plant extracts (1 ml) were allowed to react with 1 ml of the ABTS solution with 60 ml methanol to obtain an absorbance of 0.706 ± 0.001 unit where the absorbance was taken at 734 nm after 7 min using the spectrophotometer. Fresh ABTS solution was prepared for each assay. The ABTS scavenging capacity of the extract was compared with that of BHT and percentage inhibition calculated as ABTS radical scavenging activity.

The ability to scavenge DPPH and ABTS radicals were calculated by the following equation:

Radical scavenging activity (%) = [(Abs-control –Abs sample)]/(Abs-control)] × 100 where Abs-control is the absorbance of DPPH radical + methanol; Abs-sample is the absorbance of DPPH or ABTS/ radical + sample extract/standard

2.3.3. FRAP (ferric reducing ability of plasma) assay

A modified method of Adedapo *et al.* (2009) was adopted for the FRAP assay. The fresh working solution was prepared by mixing 25 ml acetate buffer, 2.5 ml TPTZ, and 2.5 ml FeCl3 6H2O. The temperature of the solution was raised to 37 ℃ before using. Plant extracts (125 µL) were allowed to react with 1425 µL of the FRAP solution for 30 min in the dark condition. Readings of the colored product (ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 M FeSO4. Results are expressed in µM Fe (II)/g dry mass and compared with that of BHT and ascorbic acid.

3. Statistical Analysis

All data were expressed as means ± S.D and were statistically analyzed using one way analysis of variance (ANOVA). Means were separated by the Duncan Multiple test using SAS (2002). Mean values were considered significant at $p<0.05$.

4. Results and Discussion

4.1. Phytochemical Content

Table 1: Phytochemical contents of the wild vegetables

Plant Species	Phytochemical constituent			
	Flavonoid mg/g	Flavonol mg/g	Pro-anthocyanidin mg/g	Phenolics mg/g
Chenopodium album	9.14 ±0.12[a]	4.48 ±0.02[a]	58.42 ±0.53[a]	9.34 ±0.12[a]
Solanum nigrum	13.30 ±0.08[b]	3.26 ±0.23[b]	63.50 ±0.01[b]	30.00 ±0.04[b]
Urtica lobulata	11.01 ±0.02[c]	3.11 ±0.01[c]	65.18 ±0.16[c]	20.25 ±0.01[c]
Amaranthus dubius	11.08 ±0.02[c]	3.18 ±0.21[b]	61.61 ±0.23[b]	18.03 ±0.42[d]

Values are means ± SD. Means followed by the same letter superscript in the same column are not significantly different from each other (P>0.05).

The phytochemical content of the wild plant species studied is presented in Table 1. Values of the total flavonoid content in the four selected plant species ranged from 9.14 in *C. album* to 13.30 µg/g dry weight in *S. nigrum*, whereas those of total phenolics were generally much higher and varied between 9.34 in *C. album* to 30.00 µg/g dry weight in *S. nigrum* (Table 1). All the plant species had a remarkably high content of pro-anthocyanidins, between 58.42 and 65.18 mg/g. in similar studies of wild edible plants Gu *et al.* (2004) [15] and Hosseinian (2007) [16], reported lower values for proanthocyanidins with the lowest at 16.4 mg/g and highest at 37.02 mg/g. These were nevertheless not the same plants but the fact that they were wild plants, it

indicates that generally the content of proanthocyanidins is high in wild plants. The highest proanthocyanidins content was measured in *U. lobulata*, but those of *S nigrum* and *A. dubius* did not differ significantly ($p>0.05$). (Table1). Results for total phenolics in *C. album and U. lobulata* are consistent to the data obtained by Afolayan and Jimoh (2009) [17] using the same procedures. Both these species had relatively high phenolics contents.

In contrast to what was previously observed in a similar study [17] of the same species but from a different region, the amounts of flavonoid and flavonol contents were low (from 0.36 to 0.98 mg/g), for the present study they were between 3.11 and 13.30 mg/g respectively. However, because some of the plants were different from those of this study, results cannot be the same. On the other hand, there was significant correlation observed for the total proanthocyanidins content in the leafy extracts of the same species in this study and Afolayan and Jimoh's work (2009) [17]. Furthermore, results that were published on the total proanthocyanidins contents in the whole fruit samples of wild berry varied from 27.55 to 50.47 mg/g [16]. These were far lower than the proanthocyanidin concentrations in the wild leafy vegetables in the present study. These contrasting findings suggest that further investigations are needed using other methods of determining these and other compounds in wild food plants. When wild plants and common vegetables were compared in respect of antioxidant content and activity, wild vegetables typically showed higher levels than cultivated common vegetables [Eaton and Konner, 1985]. Nijveldt *et al*. (2001) [18] also showed that the flavonoids and other phenolic compounds in wild and common vegetables were higher than their exotic counterparts.

These outcomes are a validation and scientific confirmation of the occurrence of these compounds in wild plants, as compounds that could contribute to the nutritive value of foods and beverages as Tulipani, *et al*., (2008) [19] observed. This study has brought to light that *C. album, S. nigrum, U lobulata* and *A. dubius,* could contribute to the nutrition security of the communities in rural areas in South Africa, as the species are common to some areas in the country. S. *nigrum* and *U. lobulata* are rich in phytonutrients such as phenols, flavonoids, flavonols and proanthocyanidins (Table 1). The phytochemical analysis indicated that analysed wild vegetables are rich sources of these compounds.

The results of the current study show a resoundingly high presence of pro-anthocyanidin in the extracts of all the plant species analysed (Table 1). The abundant occurrence of pro-anthocyanidins suggests strongly that indeed, these compounds are important components of these plant species and some of their pharmacological effects could be attributed to their presence. Pro-anthocyanidins are polymeric flavonoid compounds and are widely distributed in the plant kingdom, including plants that are important as a source of food [20]. Several studies have shown that pro-anthocyanidins have numerous pharmacological properties that benefit human health, such as *in vitro* reduction of the oxidation of human low-density lipoprotein, potent antibacterial, anti-tumor effect, anti-HIV activities and antioxidant activity [21]; (Mackenzie *et al*., 2008). Takahashi *et al*. (2001) [22] have also reported antiviral and hair-growth-promotion. Utilization of these plants for consumption would be indeed a benefit to human health.

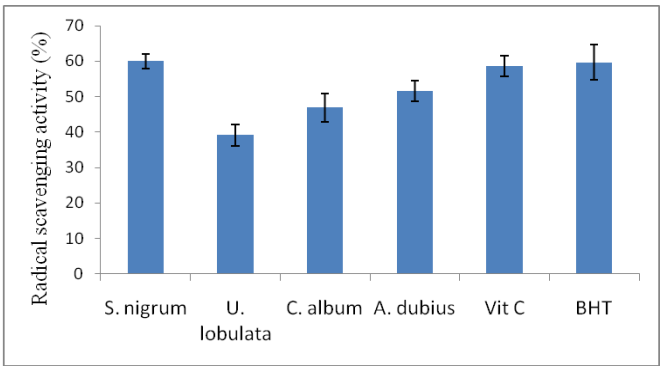

Fig. 1: DPPH free radical scavenging activity

4.2. Antioxidant Activity

4.2.1. DPPH assay

The stable radical DPPH has been used widely for the determination of primary antioxidant activity, that is, the free radical scavenging activities of pure antioxidant compounds in plant and fruit extracts and food materials [23]. The DPPH radical scavenging activity of methanolic leaf extracts of *A. dubius*, *C. album*, *S. nigrum* and *U. lobulata* measured at 517 nm as compared to standard Ascorbic acid are presented in Fig. 1. Values are the average of triplicate experiments and represented as mean ± standard deviation (SD). The highest DPPH free radical scavenging activity which was observed in *S. nigrum* (59.14%) was higher than those of both BTH and Vitamin C, while the lowest activity (39.12%) was observed in *U. lobulata*. The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability [24]. Although the DPPH radical scavenging abilities of the extracts of *A. dubius*, *C. album* and *U. lobulata* were significantly lower than those of ascorbic acid (vit C), and BHT, it was evident that the extracts did show some proton-donating ability and could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants.

4.2.2. ABTS assay

The free radical scavenging activity of the test plants along with reference standards, such as ascorbic acid were determined by ABTS assay and the results are presented in Fig. 2. The scavenging effect on the ABTS radical decreased in the order: *A. dubius* > *C. album* > *S. nigrum* > Vitamin C > *U. lobulata*. ABTS assay is commonly used to assess radical scavenging or antioxidant activity. The scavenging activity is measured by the absorbance at 414 nm, which decreased as the ABTS radical is scavenged. All of the extracts had strong antioxidant abilities that exceeded the control, Vitamin C, except *U. lobulata*. The scavenging activity of the extracts also could be due to the presence of higher levels of phenolic compounds (Table 1) which may have contributed to their high antioxidant activity as reported by Afroz *et al.*, (2006) [25].

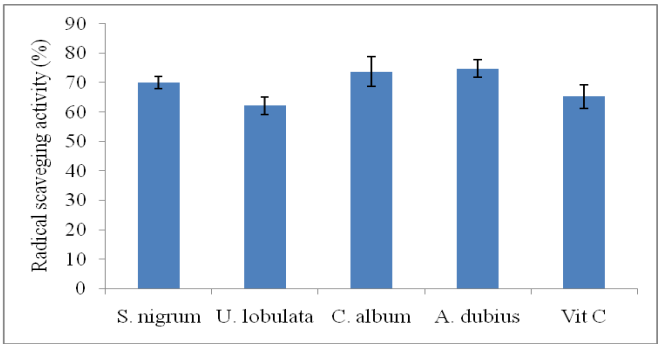

Fig. 2: ABTS free radical scavenging activity

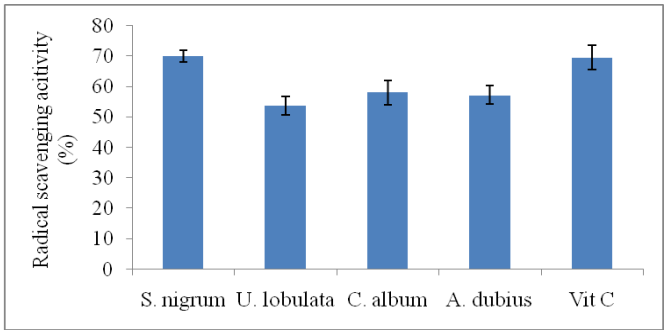

Fig. 3: FRAP assay

4.2.3. FRAP assay

The ability of the plant extracts to reduce ferric ions was determined using the FRAP assay developed by Benzie and Strain (1996). An antioxidant capable of donating a single electron to the ferric-TPTZ (Fe (III)-TPTZ) complex would cause the reduction of this complex into the blue ferrous-TPTZ (Fe (II)-TPTZ) complex which absorbs strongly at 593 nm. The trend for ferric ions reducing activities of the four plants did not vary markedly from their DPPH and ABTS free radical scavenging activities, when a comparison between the three assays was made. Similar to the results obtained for the DPPH free radical scavenging

assay; *S. nigrum* showed the strongest ferric ion reducing activities while the least activity was observed in *U. lobulata* as shown in Fig. 3. Antioxidants compounds help delay and inhibit lipid oxidation and thus maintain the nutritional quality and increase the shelf life of food when added to it. Fukumoto and Mazza (2000) [26], explained that antioxidants they tend to minimise rancidity and retard the formation of toxic oxidation products. Properly dried, the wild plants could serve as reserves during drought and recession periods and the phytochemical constituents or antioxidant agents in them, is thought to increase efficacy and minimise fungal toxicity.

5. Conclusion

This study has revealed that some wild plants should be seen to provide a lot more than just an ingredient in a diet. They possess properties capable of maintaining health. These are unique phyto-nutrients accounting for vast potential health-benefiting properties, a category of compounds representing many ecological and physiological roles, the most abundant and the most widely distributed as plant natural constituents. The results showed significantly high values for proanthocyanidin and phenolic compounds content at varying degrees in all the selected plants. The lower values registered by flavonoids and flavonols could be compensated by combining the plants when cooking. Further to this flavonoid/nols and phenolic compounds have synergistic effects when combined in a diet. Thus, the importance of interactions between various phytochemicals in reducing the risk of various degenerative diseases cannot be overlooked. It is recommended that a diet containing large quantities of fruits and vegetables including the wild food plants studied in this research should be consumed as it is where the phytochemicals/phytonutrients abound with the complete health benefits.

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