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Antioxidant and Free Radical Scavenging Activities of Selected Kenyan Leafy Vegetables

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Abstract

Naturally occurring antioxidants found in vegetables and fruits can play a critical role in deactivating free radicals often before they attack biological cells in humans. Indigenous Kenyan vegetables are potential sources of antioxidants due to their richness in phytochemicals. The study investigated free radical scavenging activities of two indigenous vegetables; Solanum nigrum (black night shade) and Gynandropsis gynandra (spider plant), and two exotic vegetables; Brassica olearacea C. (cabbage) and Brassica olearacea A. (kale), during dry and wet seasons. Fresh leaf samples were collected randomly during the two seasons. Burnt Forest (BF), Elgeyo Border (EB), Kapseret (KSS), Kesses (KS) and Moiben (MB) in Uasin Gishu County, Kenya, were used as sampling areas. Sample processing was carried out in the laboratory prior to analysis using UV-Vis spectroscopy. Radical scavenging potential values for the vegetable samples were 89% (black night shade), 79% (spider plant), 55% (kale) and 35% (cabbage). All species exhibited a 2, 2-diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity with Solanum nigrum and Gynandropsis gynandra demonstrating greater antioxidant potential. It was also observed that extracts of black night shade and spider plant exhibited higher reducing capacities than their exotic counterparts (kale and cabbage) with values ranging from 38.9±3.3 to 937.3±16.5 µmol Fe^{2+} g^{-1} for cabbage and black night shade, respectively. The order of increasing reducing capacities of samples followed the trend: cabbage, kale, spider plant and black night shade. It was concluded that indigenous vegetables demonstrated better reducing potential capacities as shown by their higher DPPH radical scavenging values compared to the exotic vegetables and this is indicative of their superior antioxidative power. The agroclimatic conditions of a sampling site also influenced the antioxidant potential of a vegetable.

Keywords: Free Radical, Scavenging Activity, Leafy Vegetables, Antioxidants

INTRODUCTION

Leafy vegetable plants of indigenous or exotic origin have traditionally been consumed by many Kenyan communities. Exotic vegetables are mainly of the *Brassica* genus and are extensively consumed worldwide. They were introduced in Kenya during colonial period and have overshadowed the native vegetables over time due to their fast growth and comparatively higher yields (Chweya & Ezyaguirre, 1999). Examples of commonly available exotic vegetables include spinach (*Spinacea oleracea*), broccoli (*Brassica*

oleracea var. Italica), cabbages (*Brassica olearacea*), cauliflower (*Brassica oleracea var. botrytis*), kale (*Brassica oleracea*), and collard greens (*Brassica oleracea L*). Prior to the introduction of exotic vegetables in the country, native vegetable species were popular. They include *Solanum scabrum* (black night shade), *Gynandropsis gynandra* (spider plant) and *Amaranthus spp*. (amaranth). Their consumption is not only confined to Kenya, but also in many other African and tropical countries (Chweya & Ezyaguirre, 1999; Akubugwo *et al.*, 2007; Mibei, 2011, Chao *et a*l., 2014; Khan *et al*., 2015).

Leafy vegetable species indigenous to Kenya have been shown to have comparable or even higher nutritional value than exotic vegetables (Grubben & Denton, 2004; Pakade, 2012; Meda *et al*., 2013). For instance, *Amaranthus spp.* and spider plant are reported to have higher metal contents than cabbage (Odhav *et al*., 2007; Orech *et al*., 2007; Kruger *et al*., 1998). Indigenous vegetables are also a rich source of vitamins and other phytochemical constituents that significantly contribute to the antioxidant activity in human diets (Gupta & Bains, 2006; Mibei, 2011). Bioactive compounds such as polyphenols and flavonoids found in high concentrations in these vegetables have beneficial health effects on humans (Francisca & Eyzaguirre, 2007). Humans are constantly exposed to harmful effects of free radicals responsible for oxidative stress and often implicated in the expression of several human diseases such as diabetes, cancer, coronary heart diseases, neurodegenerative ailments, alzheimer's disease, rheumatoid arthritis, (Pezzuto & Park, 2002; Silué, 2009), among others. Although the human body has an in-built antioxidant defence system, antioxidant-rich diets are believed to strengthen it.

Therefore, this study investigated the free radical scavenging activity of selected Kenyan leafy vegetables with antioxidant activities. The influence of spatial and temporal variability on the antioxidative capacities of these vegetables was also studied since such information remains unreported.

MATERIALS AND METHODS

Study Area

This study targeted five areas in Uasin Gishu County, Kenya, namely Burnt Forest (BF), Elgeyo Border (EB), Moiben (MB), Kapseret (KSS) and Kesses (KS). The sites were selected because they constitute the major producers of vegetables in the County and also present different agro-climatic conditions expected to influence the studied parameters. The sampling sites were systematically designated and their description provided in Table 1.

Table 1: Description of sampling sites

S/No.	Sampling site	Abbreviation	Longitude	Altitude (meters above sea level)
1	Burnt Forest	BF	00°12'55.3"N 35°25'21.5"E	2419
2	Elgeyo Border	EB	00°30'56.9"N 35°27'33.1"E	2387
3	Moiben	MB	00°40'57.2"N 35°23'32.1"E	1998
4	Kapseret	KSS	00°27'19.9"N 35°15'55.1"E	2108
5	Kesses	KS	00°17'24.8"N 35°19'55.3"E	2240

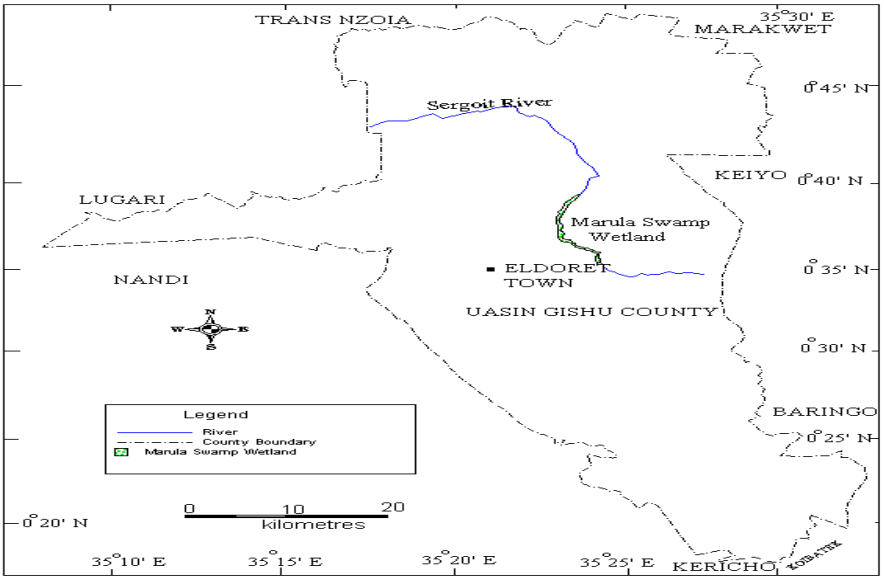

Figure 1: Location map of Uasin-Gishu County, Kenya
Source: Chebii (2014)

Sampling, Sample Treatment and Analysis
Fresh leaf samples were collected in the dry and the wet seasons of the year during the months of February and May, respectively and three samples were obtained from each site. Samples were washed with distilled water on site to remove soil and other adsorbed particulates. The samples were then air-dried and ground to fine powder.

Evaluation of Antioxidant Activity
The free radical scavenging activity of the extracts were measured using the 2,2′-diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described Brand-Williams *et al.*., (1995) with some modifications. The DPPH stock solution was prepared by dissolving 24 mg DPPH with 100 mL methanol and used immediately. Working solutions were obtained by appropriate dilution of the stock solution using methanol as a solvent. 0.1 mL of the methanolic vegetables leaves extract was added to 3.9 mL of freshly prepared 0.025 g L^{-1} DPPH solution to make a total of 4.0 mL. The reaction mixture was vigorously shaken and incubated in the dark. Decrease in absorbance was measured at 0, 5, 15, 30, 60 and 120 minutes at 515 nm. In between measurements the reaction mixtures were kept in the dark at room temperature. Methanol was used to zero the spectrophotometer. All determinations were performed in triplicate. From the data obtained, the DPPH scavenging activity was calculated using equation 1 and the IC_{50} values estimated from the percent inhibition.

$$\%DPPH_{remaining}=[(control_{absorbance}-sample_{absorbance})/(control_{asbsorbance})]\times 100 \ldots . 1$$

where, $control_{sorbance}$ is the absorbance of the control (DPPH without sample), the $Sample_{absorbance}$ is the absorbance of the test sample.

The reducing powers of the vegetables were determined according to the method of Siddhuraju & Becker (2003). Powdered extracts (0, 0.5, 1.0, 1.5, 3.0, 5.0 and 8.0 mg) of each sample was dissolved in 1 mL methanol and the resulting solution mixed with 5 mL of 0.2 M phosphate buffer (pH 6.6) and 5.0 mL of 1.0% potassium ferricyanide, $K_3[Fe(CN)_6)]$ solution. The mixture was incubated at 50°C for 20 minutes, then, 10% trichloroacetic acid (5 mL) added to each mixture, followed by

centrifugation at 5000 rpm for 10 minutes. The upper layer of the solution (5.0 mL) was extracted and mixed with distilled water (5 mL) and 1 mL of 0.1% ferric chloride added. Finally, the absorbance was read at 700 nm using UV-Vis spectrophotometer (Model 1240, Shimadzu). The assays were carried out in triplicate. Total reducing power of extracts were calculated using ascorbic acid calibration curve and expressed as mg of ascorbic acid equivalent (AAE) per 10 g of vegetables (mg AAE/10 g dw). A higher absorbance of the reaction mixture indicates greater reducing power. Data was analyzed descriptively and statistically using Pearson correlation coefficient and results are presented in figures and tables.

RESULTS AND DISCUSSIONS

Mean Antioxidant Activities of Vegetable Samples

Mean antioxidant activities of the vegetable samples measured using the remaining DPPH (%) ranged from 35% and 89% as shown in Figure 2.

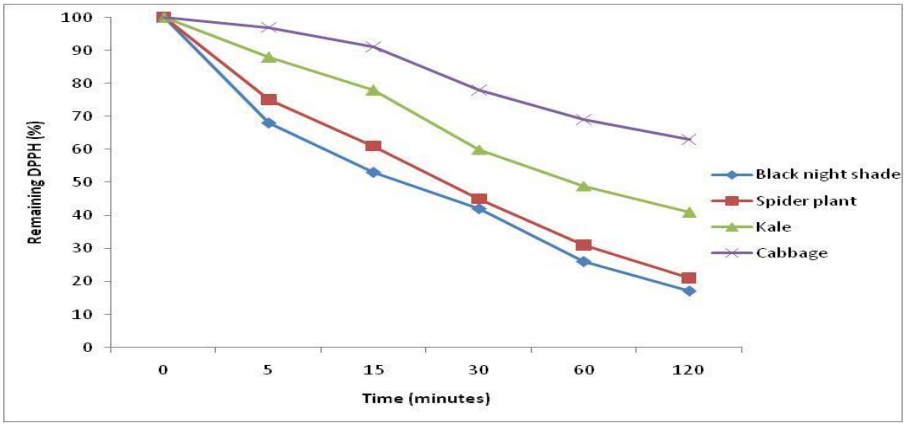

Figure 2: Antioxidant activity of vegetable extracts as % DPPH radical inhibition.

Using the amount of DPPH radicals remaining in the methanolic extracts of vegetable samples after 120 minutes, the antioxidant activities were found to follow the order: cabbage < Kales < Spider plant < black night shade. Black night shade (89%) exhibited the highest antioxidant capacity while cabbage (35 %) showed the least. The radical scavenging potential results for cabbage and kale in this study were comparable to those reported by Ferreira *et al.* (2015) in a Brazilian study where they recorded antioxidant activities of 64% and 37% for cabbage and kale, respectively. The results for kale and cabbage were also comparable to findings of Melo *et al.* (2006) who reported values of between 31 % to 78%. However, higher antioxidant capacities of black night shade and spider plant (native vegetables) as compared to cabbage and kale was recorded. Radical scavenging activities of black night shade and spider plant vegetables were found to be comparable but those between the indigenous and the exotic vegetables were statistically significant ($P \leq 0.05$).

Spatial Variability in the Antioxidant Activities of Vegetable Samples

Results for free radical scavenging activities of black night shade and spider plant samples as affected by source are presented in Figure 3. A gradual reduction in amounts of the DPPH free radicals was evident during the 120 minute exposure period with samples from some sites producing steeper declines than others. Concentration of DPPH free radicals remaining in solution after the exposure period for black night shade and spider plant extracts followed the trend MB < KS < EB < BF < KSS, and MB < BF < KS < EB < KSS, respectively. Antioxidant activities of black night shade

and spider plant as shown by percent DPPH inhibition ranged from 73% to 85%, and 70% and 82%, respectively. Black night shade yielded a 21.8% mean value of DPPH radicals remaining in solution, while spider plant had 24.2% of DPPH not scavenged. The difference in the antioxidant activity values between the two native vegetables was marginal and not statistically significant ($P \leq 0.05$). It was observed that for both vegetables, samples from site MB showed the strongest antioxidant activity (15% for black night shade and 18%), while KSS had the least (27% and 30% for black night shade and spider plant, respectively).

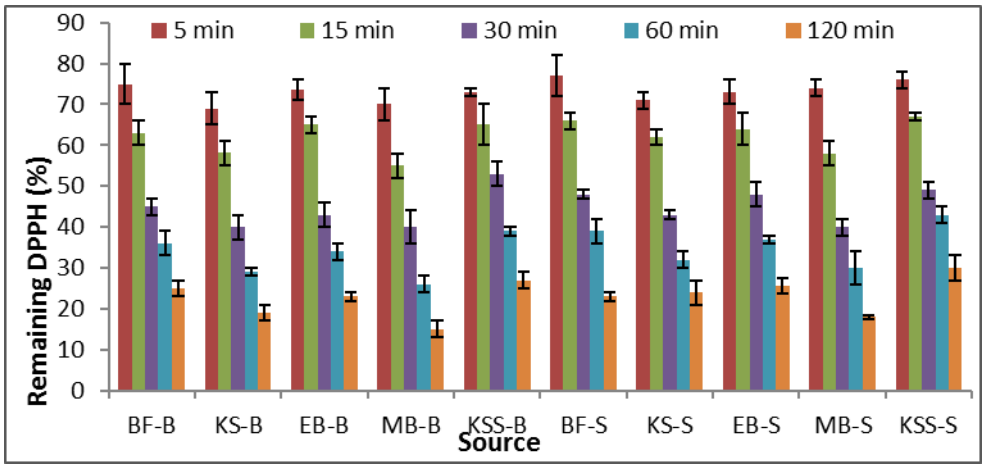

Figure 3: Effect of source on the antioxidant activity of Black Night Shade and Spider Plant vegetables.

Temporal Variability in the Antioxidant Activities of Vegetables

The temporal variability in the free radical scavenging activity of black night shade and spider plant vegetable samples are shown in Figures 4 and 5. Black night shade methanolic extracts demonstrated significant seasonal differences in their ability to scavenge DPPH radicals, exhibiting a wide range of scavenging values from 88% at MB in the dry season to 68% at KSS in the wet season. A similar observation was made for spider plant extracts where radical scavenging values ranged from 81% (MB, dry season) to 50% (KSS, wet season). Mean seasonal antioxidant activity values for black night shade extracts was 83.8% in the dry season and 77.6% in the wet season, while spider plant yielded 76.8% and 63.4% for dry and wet seasons, respectively. A mean antioxidant activity difference of 27.7% between the dry and wet season samples was found in black night shade while a value of 34.6 % was obtained for spider plant.

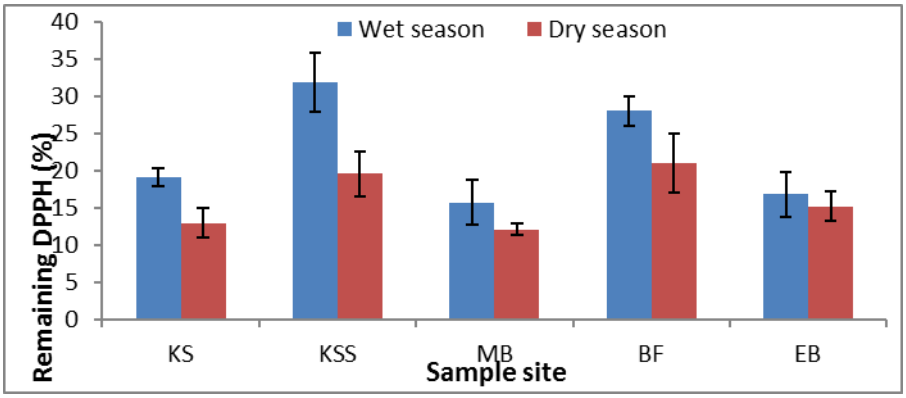

Figure 4: Temporal variation in the free radical scavenging activity of Black Night Shade samples.

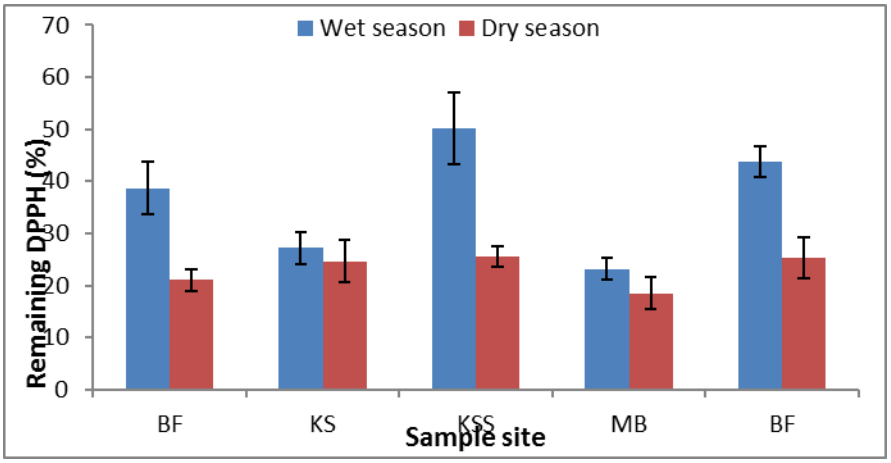

Figure 5: Temporal variation in the free radical scavenging activity of Spider Plant samples.

Reducing Power of the Vegetable Extracts

The reducing powers of the vegetable samples based on the extract's ability to provide electrons for reduction of the iron (III) to iron (II) ions in solution are presented in Table 1 and Figure 6.

Table 2: Reducing power of the vegetable extracts

Vegetable type	Reducing power (µmol Fe^{2+} g^{-1})
Black night shade	937.3±16.5
Spider plant	765.1±11.8
Kale	356.5±9.6
Cabbage	38.9±3.3

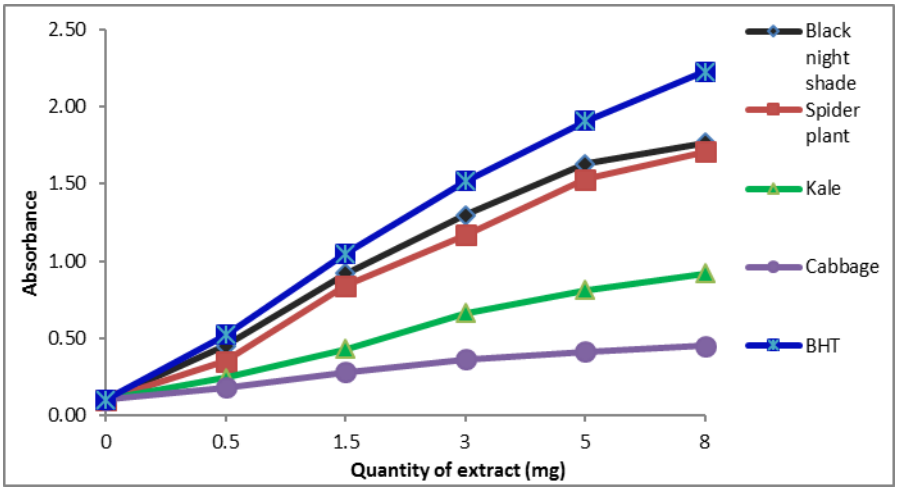

Figure 6: Reducing power of the various vegetables as affected by amount of extract (BHT = Butylated hydroxyanisole).

It was observed that extracts of black night shade (937.3±16.5 µmol Fe^{2+} g^{-1}) and spider plant (765.1±11.8 µmol Fe^{2+} g^{-1}) exhibited higher reducing capacities than kale (356.5±9.6 µmol Fe^{2+} g^{-1}) and cabbage (38.9±3.3 µmol Fe^{2+} g^{-1}). The reducing capacities of the vegetables followed the order: cabbage < kale < spider plant < black night shade. No significant difference ($p \leq 0.05$) was observed between the two native vegetables but a significant difference ($p \leq 0.05$) was evident between the indigenous and exotic vegetables. Kale and cabbage samples showed the weakest reducing power and the difference between these two vegetables was significant. Mitic *et al.* (2013) also reported a similar observation where cabbage showed the lowest reducing activity of 9.455 µmol Fe $10g^{-1}$ fw.

Methanolic extracts of the four vegetable samples also clearly displayed a dose-dependent reducing power with a threshold value at around 5 mg mL^{-1} for black night shade, spider plant and kale vegetable extracts, as shown by the leveling off of the graphs (Figure 5). However, cabbage extracts leveled off at a concentration of around 3 mg mL^{-1} while the reducing power for the standard, butylated hydroxyanisole (BHT), used for comparison had not shown signs of leveled off at a concentration of 8 mg mL^{-1}.

Effect of Source and Seasonal Variability on the Reducing Power of the Vegetable Extracts

The mean reducing power findings for black night shade and spider plant vegetables sourced from the various study sites are presented in Figure 7 while Figure 8 presents their seasonal variability. The highest capacity to reduce Fe^{3+} to Fe^{2+} was found in samples from site MB while the lowest was from KSS. The order of sample sites ranked as follows: MB > KS > EB > BF > KSS. However, the difference in the reducing power between vegetable samples from the various sites was not significant ($p \leq 0.05$). The vegetable samples showed distinct seasonal variation in their abilities to reduce Fe^{3+} to Fe^{2+} ions in reagent solution. The reducing power of black night shade averaged 1162.3 µmol Fe^{2+} g^{-1} dw in the dry season and 712.3 µmol Fe^{2+} g^{-1} dw in the wet season. On the other hand, spider plant yielded average reducing power values of 994.6 and 535.4 µmol Fe^{2+} g^{-1} dw in the dry and wet seasons, respectively.

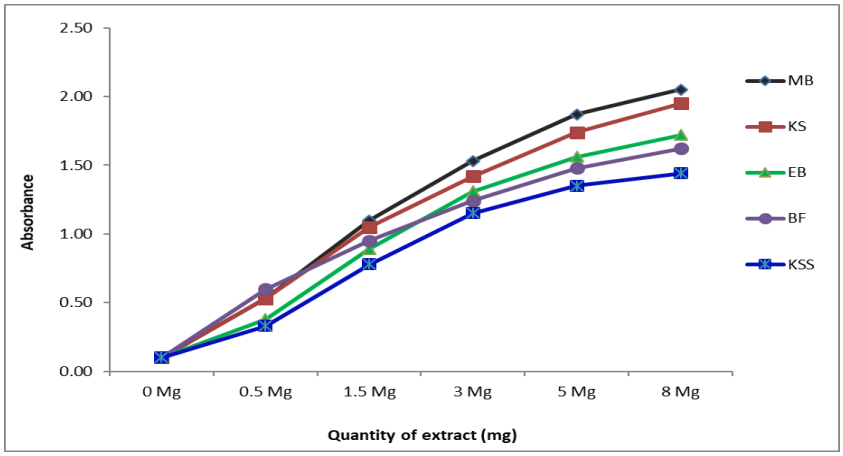

Figure 7: Spatial variability in the reducing power of Black Night Shade and Spider Plant vegetable samples.

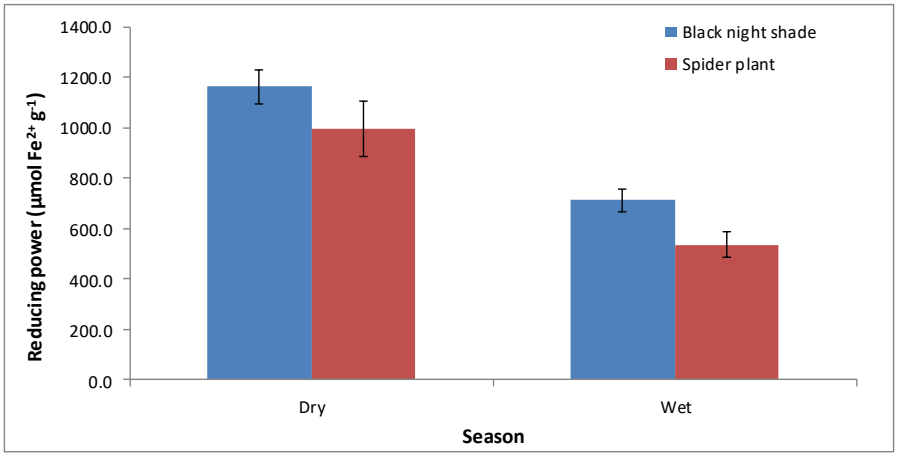

Figure 8: Seasonal variability in the reducing power of Black Night Shade and Spider Plant vegetables.

Antioxidant Activity Variations

Biological molecules in the body are subject to attack by free-radical causing damage (to DNA and membrane lipids) and cell impairment/death, eventually leading to disease conditions such as cancer, cardiovascular disease, rheumatoid arthritis, diabetes, and neurological disorders (Valko *et al.*, 2007). Antioxidants, mainly derived from dietary constituents are known to neutralize such free radicals or their actions (Mibei, 2011). The antioxidant activities of the vegetable extracts were investigated using DPPH reagent. DPPH is a very stable free radical generation system with the advantage of not being affected by certain side reactions such as metal ion chelation and enzyme inhibition (Huyut *et al.*, 2017). The lower the concentration of DPPH free radicals remains in solution after the exposure period, the higher is the ability of that particular extract to scavenge free radicals. The technique is based on the antioxidant species reducing the purple-colored 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical (absorbs at 517 nm) to the yellow-coloured 2,2-diphenyl-1picrylhydrazyl with subsequent disappearance of absorption making it possible to monitor decrease in absorbance.

Typically, the ability of a species to act as an antioxidant depends on its chemical structure and the ease in which it donates or accepts electrons, thus delocalizing an unpaired electron within an aromatic structure. From the results, it is possible to obtain the free radical antioxidant or scavenging activity percentage and/or the DPPH remaining percentage in the reaction environment.

The difference in concentration of total antioxidant activity in a plant varies widely and is influenced by several factors that include climatic conditions (Rodriguez-Amaya, 2003) prevalent in an area where the plant is growing. In the present study, it was apparent that vegetables grown in areas that experience more adverse climatic conditions such as, higher temperatures, more sunlight hours, stronger UV radiation and water stress tended to have elevated antioxidant activity compared to sites that enjoyed milder conditions. This may explain the stronger antioxidant activity exhibited by vegetables from sites such as MB and KS as opposed to BF and EB. Synthesis of secondary metabolites is influenced by ecological factors such as water stress, UV exposure and temperature (Masa *et al.,* 2016) for the purpose of protecting the plant. In particular, hydric stress experienced in the dry season induces stomatal closure thereby decreasing carbondioxide (CO_2) concentration in leaf mesophyll tissue resulting in an accumulation of nicotinamide adenine dinucleotide phosphate, NADPH (Odhiambo, 2015). Such conditions, where NADPH is a limiting factor, encourages formation of superoxide radical ($O_2^{\bullet}$) where oxygen acts as an alternate acceptor of electrons from the thylakoid electron transport chain (Cadenas, 1989). Superoxide radical and its reduction product (H_2O_2) are potentially harmful compounds, and can also combine by the Haber-Weiss reaction to form the highly toxic hydroxyl radical ($OH^{\bullet}$.) (Sairam *et al.,* 1998). Therefore, plants react by increasing production and storage of antioxidant compounds and their metabolites making it possible to neutralize the reactive oxygen species (ROS), thus reducing the effect of oxidative damage.

Seasonal variability of the reducing capacities of the samples generally adopted a similar trend as those of DPPH activity, TPC, TFC and flavonol values indicating that the climatic conditions of a location may be an important determinant of the antioxidant amounts in a plant material and hence, its reducing capacity. Since site MB experiences relatively more severe climatic conditions, synthesis and accumulation of antioxidant compounds is more pronounced, thereby increasing the vegetables' reducing properties. An opposite argument may be advanced for samples obtained from BF and EB that experience milder climatic conditions. Indeed, in a study of wild artichoke vegetables, Hubber *et al.* (2008) reported that the plants native to Slovakia showed stronger ability to reduce Fe^{3+} (350 µmol Fe^{2+} g^{-1}) compared to those native to Brazil (98.7 µmol Fe^{2+} g^{-1}), an observation attributable to differences in climatic conditions between the two regions of the world. Pakade (2012) also made a similar observation on the flavonol content in *Moringa* plants grown in several regions of South Africa.

Therefore, the dry season produced higher mean sample reducing capacities than the wet season for both vegetables, in agreement with the other reported parameters (such as TPC, TFC and flavonol amounts). Usually, reducing capacity of a compound may serve as an important indicator of its potential antioxidant activity (Chao *et al.,* 2014) and these values have seasonal dependencies.

CONCLUSION AND RECOMMENDATIONS

Conclusion

The DPPH radical scavenging values gave figures that were between 35% to 81% and the indigenous species proved to have better antioxidant capacities than their exotic counterparts as shown by the strong positive

and significant correlation ($r = 0.6429$ to $r = 0.7280$, $p < 0.05$) between the amount of phenolics in the vegetable extracts with the DPPH antioxidant activity. This was a good indicator of their antioxidative potential. Results were influenced by climatic condition of each region. Temporal variation was significant especially during the dry season where season samples produced higher amounts of target analytes. From the results its apparent therefore that indigenous vegetables studied is rich in free radicals scavenging capabablilities and should be highly encouraged for consumption than exotic vegetables. The study recommends toxicological analysis of these two plants to be undertaken with a view of including them in the list of potential medicinal and nutritional sources in the community.

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