

Antioxidant potential of the farmer preferred selections of *Solanum aethiopicum* vegetable consumed in central Uganda

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ABSTRACT

In addition to the rich micronutrient value, indigenous vegetables are regarded as possessing medicinal attributes. The Solanaceae family has over 1000 species worldwide, with a number of indigenous species originating in Africa. The most popular leafy vegetable in Uganda is the *Solanum aethiopicum* (Nakati). The objective of this study was to determine the selected phytochemical attributes, chlorophyll content, moisture content and total antioxidant activity of the farmer preferred selections within the landraces of *Solanum aethiopicum* leafy vegetable in Uganda. The antioxidant activity was achieved by screening the leaf extracts for their free radical scavenging properties using diphenyl picryl hydrazyl (DPPH) and ascorbic acid as standard. The ability of the extracts to scavenge DPPH radical was determined spectrophotometrically at 517 nm. The study showed

that all the landraces had a high polyphenol and flavonoid content with SAS185/P/2015 containing the highest flavonoid content (3.16±0.06 mg QE/g fw). SAS1641/2015 showed the highest total polyphenol content of 7.79±0.27 mg GAE/g fw and also showed the highest vitamin C content. This contributed to the high total antioxidant activity of 2.79±0.01 and 5.43±0.02 mg AAE/g fw when using FRAP and DPPH methods respectively. SAS145/2015 presented the highest chlorophyll content of 19.69±0.01 mg/g dwb. All the landraces showed a high percentage moisture content that ranged from 82.66±0.35 to 84.21±0.48%. These results are of nutraceutical significance and hence confirm their usage as medicinal vegetables.

Keywords: Landraces; Polyphenols; Flavonoids; Vitamin C; Total antioxidant activity; Ferric reducing antioxidant power (FRAP); Diphenyl-picrylhydrazyl (DPPH).

1. INTRODUCTION

Consumption of fruits and vegetables has attracted growing interest because many epidemiological and biochemical studies have consistently demonstrated a clear and significant positive association between intake of these natural food products, and reduced rates of chronic diseases such as heart disease, common cancers, degenerative diseases and as well as aging [1]. The protection that fruits and vegetables provide against these maladies is attributed to the presence of several antioxidants such as ascorbic acid (vitamin C), α -tocopherol (vitamin E) and β -carotene (provitamin A) [1, 2] and polyphenolic substances [1].

Vegetables in Uganda are mostly grown by small scale farmers at subsistence levels. There are over 600 local vegetable species in Uganda [3]. These vegetables are perishable and low yielding and their commercial value has not yet been well explored. The traditional vegetables have very high nutritious value [4] for example they are rich in β -carotene, vitamins E and C, proteins and minerals such as iron, calcium, phosphorus, iodine and fluorine. Most traditional vegetables have medicinal value for example *Solanum indicum* (Katunkuma) is used to control high blood pressure, *Amaranthus dubius* (dodo) and *Amaranthus lividus* (ebuga) are also believed to increase blood levels [3, 5]. The consumption of vegetables has been known to alleviate micronutrient malnutrition which is the cause of chronic diseases [5]. The most common traditional vegetables grown especially in central Uganda include *Amaranthus dubius*, *A. lividus*, *A. blitum* (Ebugga eryanamusayi); *Solanum aethiopicum* (Nakati); *S. gilo* (Entula enganda); *S. indicum* subsp. *distichum* or *S. anguivi* (Katunkuma); *S. nigrum* (Ensugga enzirugavu); and *Gynandropsis (Cloeme) gynandra* (Ejjobyo) [3]. These leafy vegetables are used as side-dish accompanying the thick starchy meals [5]. Only a few of these vegetables are commercially grown.

There are over 1000 species of the Solanaceae family out of which 100 indigenous species are in Africa [6] and several studies have supported the use of these vegetables as foods and medicinal preparations [6]. Despite these benefits from vegetables and the nutrients they contain, there has been limited research on local vegetable varieties in

Uganda [3]. Of the commonly grown vegetable varieties, *Gynandropsis (Cloeme) gynandra*, *Amaranthus dubius*, *A. lividus*, *A. blitum* and *Solanum aethiopicum* are grown by a larger number of farmers and ranked higher than the others, for both food and cash. *Solanum aethiopicum* is the most commonly grown leafy vegetable in Uganda. Within this vegetable, there are several landraces which differ in stem color, leaf color (with different shades of green), leaf margin structure, leaf size and stem height at market maturity. Farmers in Uganda prefer *Solanum aethiopicum* with broad leaves, 1.5 feet height at market maturity and that with strong green color. These differences may be due to genetics and environmental factors. Due to the differences, there is expected difference in the phytochemical attributes within the landraces. Many of these selections are mainly consumed for their nutritional values and the acceptability of these vegetables depends on texture and appearance that depends on the chlorophyll content [7] without much consideration for their therapeutic importance. Few vegetables in Uganda have been explored for phytochemical and antioxidant activity studies. The objective of this study was therefore to determine the selected phytochemical attributes, total antioxidant activity, vitamin C, chlorophyll and moisture content of the farmer preferred selections within the landraces of *Solanum aethiopicum* leafy vegetable.

2. MATERIALS AND METHODS

2.1. Chemicals and reagents

The chemicals and reagents used included: methanol, Folin-Ciocalteu, sodium hydrogen carbonate, gallic acid, aluminium chloride, Na-K tartarate, quercetin, diphenylpicrylhydrazyl, 2,4,6-tri(2-pyridyl)-s-triazine, iron(III) chloride, ascorbic acid, metaphosphoric acid, glacial acetic acid, 2,4-dinitrophenyl hydrazine, hydrochloric acid, ammonium thiocyanate and acetone. They were all of analytical grade and purchased from Sigma Germany. The filter papers were purchased from Whatman UK.

2.2. Sample treatment

The screened landraces were collected from

the Uganda Christian University Agricultural research Farm in Ntawo, Mukono district, central Uganda. The whole *S. aethiopicum* plants were uprooted, shaken to remove soil and wrapped in aluminium foil, put in a cooler box and transported to the research laboratory of the Food Technology and Nutrition department of the college of Agricultural and Environmental sciences, Makerere University, Uganda. The edible parts of the plant (leaves) were removed and washed with running water and used for extraction to determine content of selected phytochemicals and total antioxidant activity except for samples used for moisture content determination which were not washed.

2.3. Preparation of extracts

The extraction was done according to the method previously described by Wissam et al. [8] but with modification. The edible parts of fresh leaves were blended and 1 g of powdered sample dissolved in 50 ml of 80% methanol solution in a conical flask placed in a thermostatic water bath shaker at 45°C for 20 minutes. The liquid extract was separated from solids by centrifugation at 2000 rpm for 10 minutes and the supernatant stored at -20°C. This extraction was done in triplicates.

2.4. Determination of polyphenol content

The total phenolic content was determined by Folin-Ciocalteu's method [9]. This was done by measuring 0.5 ml of extract, and adding 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water, followed by 2.5 ml of 7.5% NaHCO₃, incubated at room temperature in the dark for 45 minutes and absorbance was determined at 765 nm. The samples were prepared in triplicate for each analysis and mean values of absorbance obtained. The standard solutions of gallic acid of concentrations 0.01, 0.02, 0.03, 0.04, 0.05 mg per ml were used to construct a standard calibration curve [10] and the concentration of phenolics were determined in mg per g of fresh sample.

2.5. Determination of flavonoids content

The flavonoid content was determined using the aluminium chloride method [11]. To the aliquots

of extract solution made up to 3 ml with methanol, 0.1 ml of 10% AlCl₃ solution, 0.1 ml Na-K tartarate and 2.8 ml of distilled water was added sequentially and mixture shaken vigorously and incubated for 30 minutes at room temperature in the dark and absorbance determined at 415 nm using Genesys 10-UV spectrophotometer (Thermo Electron Corporation, Madison WI, USA). Known concentrations of quercetin, 0.01, 0.02, 0.03, 0.04, 0.05 mg per ml were used to generate a standard calibration curve for absorbance at 415 nm. Then concentration of flavonoids calculated from calibration curve and expressed as mg quercetin equivalent per g of fresh sample.

2.6. Determination of antioxidant activity

2.6.1. Free radical scavenging activity by the DPPH method

The free radical scavenging activity was assayed using free radical scavenging activity via DPPH method as previously described [12]. DPPH stock solution (1M) in methanol was prepared and kept at -20°C and a 0.1 mM DPPH was used for the test which was prepared by diluting 10 ml of the stock solution with 90 ml of methanol. Ascorbic acid was used as the standard prepared with concentrations of 25, 50, 75, 100 and 125 µg/ml. Equal volumes of 1.5 ml of the standard and the sample was added and kept in the dark for 30 minutes and absorbance measured at 517 nm using Genesys 10-UV spectrophotometer (Thermo Electron Corporation, Madison WI, USA). The percentage inhibition of both standard and samples calculated.

$$\% \text{ inhibition} = [(AB - AA) / AB] \times 100$$

AB is absorbance of control sample and AA is absorbance of sample.

A calibration curve was obtained by plotting % inhibition against ascorbic acid concentration. The results were expressed as ascorbic acid equivalent in mg/g of fresh sample.

2.6.2. Radical scavenging activity by FRAP method

Ferric reducing antioxidant power assay was performed using the method as previously described but with modifications [13]. To the extract (1 ml)

was added with 1 ml of FRAP reagent, that was prepared with mixture of 300 mM sodium acetate buffer (pH 3.6), 10 mM 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ) solution and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ in a ratio of 10:1:1, and diluted with water to a total volume of 4 ml. The reaction mixture was incubated in a water bath at 37 °C for 30 minutes and absorbance determined at 593 nm using Genesys 10-UV spectrophotometer (Thermo Electron Corporation, Madison WI, USA). Ascorbic acid was used as the standard prepared with concentrations of 25, 50, 75, 100 and 125 µg/ml. The results were expressed as ascorbic acid equivalent in mg per g of fresh sample.

2.7. Vitamin C analysis

Extraction method for vitamin C extraction was done using 5% metaphosphoric acid - 10% acetic acid solution [14]. The fresh leaves of vegetable were blended and to 10 g of the blended sample, 50 ml of 5% metaphosphoric acid - 10% acetic acid solution added and mixture transferred into a 100 ml volumetric flask and shaken gently until a homogeneous dispersion is obtained. The mixture was diluted to the mark with 5% metaphosphoric acid - 10% acetic acid solution. The resultant mixture was filtered using Whatman No. 1 filter paper and filtrate used to determine the total Vitamin C in the sample. This was repeated every after 24 hours for seven days for every postharvest handling method. The extraction was done in triplicates. The total vitamin C was determined using UV-spectrophotometer [14]. Excess bromine water was added to 1 ml of extract (bromine oxidizes ascorbic acid to dehydroascorbic acid), 3 drops of thiourea were added to remove excess bromine to form a colorless solution followed by 1 ml of 2,4-dinitrophenyl hydrazine to form an osazone and mixture incubated at 37°C for 3 hours in a water bath. The mixture was finally cooled in an ice bath and 5 ml of 85% sulphuric acid added with constant stirring to obtain a red colored complex and absorbance determined at 521 nm using Genesys 10-UV spectrophotometer (Thermo Electron Corporation, Madison WI, USA). A 0.5 mg/ml solution of L-ascorbic acid stock solution was prepared and used to prepare different standard solutions of ascorbic acid which were used to

develop a standard curve from which the vitamin C concentrations in the leaves was determined.

2.8. Determination of chlorophyll

The chlorophyll was extracted with acetone and determined using a UV Vis spectrophotometer [15]. Accurately weighted 0.1 g of fresh leaf sample was taken, and macerated in 10 ml of 80% acetone solution as extracting solvent using celite. The mixture was then filtered using Whatman No 1 filter paper and filtrate diluted with 80% acetone. The solution mixture was then analyzed for Chlorophyll-a and Chlorophyll-b content in a UV-spectrophotometer at 663.2 nm and 646.8 nm respectively using Genesys 10-UV spectrophotometer (Thermo Electron Corporation, Madison WI, USA). The quantification of chlorophyll a and b was done using the equation described shown below and results expressed in mg/g dwb.

$$\text{Cha} = 12.25A_{663.2} - 2.79A_{646.8}$$

$$\text{Chb} = 21.5A_{646.8} - 5.1A_{663.2}$$

2.9. Determination of moisture content

The moisture content of the samples was determined as previously described [16]. A thoroughly washed Petri-dish was placed in the oven to dry and then weighed. The blended sample (3 g) was then placed in the weighed Petri dish, and then placed in an oven to dry at 60°C for 16 hours. The dish and dry sample were transferred to a desiccator to cool at room temperature before being weighed again. Every sample was analyzed in triplicate.

2.10. Statistical analysis

The results were reported as the mean and standard deviation. Analysis of variance (ANOVA) was applied to the data using SPSS version 16.0 for windows (SPSS, Inc., Chicago, IL, USA). The significant differences were obtained using the Tukey HSD test ($p \leq 0.05$) and correlation coefficients between antioxidant components and antioxidant activity were determined.

3. RESULTS

3.1. Total polyphenols and flavonoids content

The total flavonoid content was ranging from 1.91 ± 0.16 to 3.16 ± 0.06 mg/g fw of fresh sample. There was a significant difference ($P \leq 0.05$) in the flavonoid content among the six landraces. SAS185/P/2015 showed the highest amount of flavonoid content (3.16 ± 0.06 mg QE/g fw) followed by SAS184/G/2015 (2.68 ± 0.04 mg QE/g fw) and SAS137/P/2015 (1.91 ± 0.16 mg QE/g fw) contained the least amount of flavonoids as shown in Table 1. The total polyphenol content ranged from 3.44 ± 0.11 to 7.79 ± 0.27 mg GAE/g fw. Total polyphenol content was significantly different ($P \leq 0.05$) among the landraces with SAS1641/2015 (7.79 ± 0.27 mg GAE/g) showing the highest total polyphenol followed by SAS185/P/2015 (6.61 ± 0.15 mg GAE/g fw) and SAS145/2015 (3.44 ± 0.11 mg GAE/g fw) was observed to contain the least amount of total polyphenols as shown in Table 1.

3.2. Vitamin C

The vitamin C content as determined using UV-spectrophotometric method showed that SAS1641/2015 (1.9 ± 0.04 mg/g fw) had the highest content of vitamin C followed by SAS184/G/2015 (1.53 ± 0.15 mg/g fw) and SAS137/P/2015 (0.52 ± 0.16 mg/g fw) showed the least content of vitamin C as shown in Table 1. The results showed a significant difference ($P \leq 0.05$) in the vitamin C content among the landraces studied.

3.3. Chlorophyll content

The chlorophyll extracted using 80% acetone was determined by UV-spectrophotometer. The results showed a significant difference ($P \leq 0.05$) in the chlorophyll content of the landraces. The highest chlorophyll content was observed in SAS145/2015 (19.69 ± 0.01 mg/g dwb) followed by SAS137/P/2015 (19.54 ± 0.13 mg/g dwb) and SAS1641/2015 (17.94 ± 0.003 mg/g dwb) showed the least observed chlorophyll content.

3.4. Moisture content

High percentage moisture content was observed ranging from 82.34 ± 0.28 to $84.21 \pm 0.48\%$. The percentage moisture content of SAS148/G/2015 ($82.34 \pm 0.28\%$) was significantly different from the percentage moisture content of the other landraces except SAS1641/2015 ($82.66 \pm 0.35\%$) at $P \leq 0.05$. SAS185/P/2015 ($84.21 \pm 0.48\%$) had the highest observed percentage moisture content among all the six landraces. There was no significant difference in the percentage moisture content of the other five landraces studied as shown in Table 1.

3.5. Total antioxidant activity

The total antioxidant activity was determined using FRAP and DPPH method. The two methods showed that SAS1641/2015 had the highest total antioxidant activity of 2.79 ± 0.01 and 5.43 ± 0.02 mg AAE/g of fresh sample with FRAP and DPPH method respectively as shown in Table 2.

Table 1. Phytochemical content and moisture content of the farmer preferred landrace of *Solanum aethiopicum* Sham.

Accession number	Flav (mgQE/gfw)	T.P (mgGAE/gfw)	Total Vit. C (mg/gfw)	Chl (mg/gdwb)	Moisture %
SAS145/2015	2.30 ± 0.04^a	3.44 ± 0.11^a	1.36 ± 0.28^a	19.69 ± 0.01^a	83.49 ± 0.33^a
SAS148/G/2015	2.37 ± 0.02^b	5.04 ± 0.43^b	0.60 ± 0.02^b	19.09 ± 0.00^b	82.34 ± 0.28^b
SAS1641/2015	2.50 ± 0.09^{abc}	7.79 ± 0.27^c	1.90 ± 0.04^{ac}	17.94 ± 0.00^c	82.66 ± 0.35^{ab}
SAS185/P/2015	3.16 ± 0.06^c	6.61 ± 0.15^d	1.50 ± 0.10^{ac}	19.58 ± 0.11^a	84.21 ± 0.48^a
SAS184/G/2015	2.68 ± 0.04^c	4.38 ± 0.22^e	1.53 ± 0.15^{ac}	18.26 ± 0.03^d	83.86 ± 0.12^a
SAS137/P/2015	1.91 ± 0.16^d	4.80 ± 0.08^{be}	0.52 ± 0.16^b	19.54 ± 0.13^a	83.59 ± 0.08^a

Flav; flavonoids, DPPH; 1,1-diphenyl-2-picrylhydrazyl, FRAP; Ferric Reducing Antioxidant Power, T.P; Total Polyphenols, Total Vit C; Total Vitamin C, Chl; Chlorophyll content. Values are expressed as means \pm standard deviation. ^{abcde} Values not sharing common superscript with in a column are significantly different ($P \leq 0.05$) using Tukey HSD test.

Table 2. Total antioxidant activity determined using FRAP and DPPH method, of the farmer preferred landrace of *Solanum aethiopicum* Sham.

Landrace	Total antioxidant activity (mg AAE/g fw)	
	FRAP assay	DPPH assay
SAS145/2015	1.55±0.06 ^a	5.20±0.04 ^a
SAS148/G/2015	2.56±0.03 ^b	5.05±0.01 ^b
SAS1641/2015	2.79±0.01 ^c	5.43±0.02 ^c
SAS185/P/2015	2.66±0.03 ^{bc}	5.15±0.02 ^{ab}
SAS184/G/2015	1.53±0.08 ^a	3.84±0.07 ^d
SAS137/P/2015	1.66±0.14 ^a	4.24±0.05 ^e

Values are expressed as means ± standard deviation. ^{abcde} Values not sharing common superscript within the same column are significantly different ($P \leq 0.05$) using Tukey HSD test.

Table 3. Pearson correlation coefficient for the selected parameters and total antioxidant methods used.

Variable	DPPH	Flav	T.Ps	FRAP	Vit. C	Moisture	Chl
Flav	0.212						
T.P	0.475*	0.409					
FRAP	0.673**	0.450	0.851**				
Vit. C	0.294	0.602**	0.441	0.233			
Moisture	-0.398	0.381	-0.213	-0.400	0.194		
Chl	0.099	-0.146	-0.466	-0.260	-0.531*	0.354	
Phytates	-0.152	0.637**	0.284	0.285	-0.051	0.571*	0.294

Flav; flavonoids, DPPH; 1,1-diphenyl-2-picrylhydrazyl, FRAP; Ferric Reducing Antioxidant Power, T.Ps; Total Polyphenols, Vit. C; Vitamin C, Chl; Chlorophyll content. ** and * Correlation is significant at the 0.01 and 0.05 level respectively.

This high total antioxidant activity may have been due to the high total polyphenol, flavonoid and vitamin C content as shown in Table 1. The lowest total antioxidant activity was observed in SAS184/G/2015 (1.53±0.08 and 3.84±0.07 mg AAE/g of fresh sample with FRAP and DPPH methods respectively).

4. DISCUSSION

The phytochemical content of vegetables is determined by the presence and activation of key enzymes for example phenylalanine ammonia-lyase, γ -tocopherol methyltransferase, l-galactose dehydrogenase which are responsible for the biosynthesis of polyphenols, α -tocopherol, and ascorbic acid respectively [17, 18]. The significant difference in the phytochemical attributes and moisture content of

the landraces of *Solanum aethiopicum* is attributed to the genetic differences in the respective landraces [19]. The high antioxidant activity of SAS1641/2015 is due to its high flavonoid, total polyphenol and vitamin C content as shown in Table 1. This is explained by the positive correlation of flavonoid content, total polyphenol content and vitamin C content showed with the total antioxidant activity when determined using both FRAP and DPPH methods. This positive correlation is previously demonstrated by other researchers [20]. The high total antioxidant activity was mainly contributed by the polyphenols as shown in Table 3, as total polyphenols have significant correlation at 0.01 level with total antioxidant activity determined using FRAP method and with that when using DPPH method at 0.05 level. *Solanum aethiopicum* is a green leafy vegetable with high moisture content

as results show in Table 1. This has also been shown in other studies done earlier on indigenous vegetables [21-24]. The high chlorophyll content of all the landraces increases the acceptability of this vegetable since this depends on appearance and texture [7].

5. CONCLUSION

The present research provides for the first time a report on the phytochemical qualities and antioxidant activity of selected landraces of *S. aethiopicum* preferred by farmers. All *S. aethiopicum* landraces studied had a high content of polyphenols and antioxidant activity. This makes *S. aethiopicum* an important plant for the control of diseases like cancer, diabetes mellitus and heart diseases. Ugandans should be encouraged to consume these vegetable in order to avert oxidative stress related diseases. The high percentage moisture content of *S. aethiopicum* explains its short shelf life and calls for a proper storage technology if it is to be consumed fresh. Within the landraces are physiological and biochemical differences that result in differences in phytochemical content. There is need therefore for a study on the genetic differences of the different landraces of *S. aethiopicum* and how the genetics affects the phytochemical content and shelf life of the landraces and also develop a storage technology that can preserve the chlorophyll content and regulate the yellowing effect of ethylene.

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AUTHOR'S CONTRIBUTION

SS: Developing and designing the concept, developing the methodology, collecting, analysis and interpretation of data and writing, review and revision of the manuscript. A Nandutu: Involved in the developing, designing the concept, developing the methodology, analysis and interpretation of data, provided technical support and involved in the study supervision, review and revision of the manuscript. A Namutebi: Developing and designing

the concept, developing the methodology, analysis and interpretation of data, provided administrative, technical and material support, involved in the study supervision. JS: involved in acquisition of data and administration. EBK: was involved in the development and design of the concept, screening of the landraces and provided administrative, technical and material support. PK: Involved in the screening of the landraces, development of the concept and acquisition of data. JNJ: Involved in the administration of the project. AK: Involved in the screening of the landraces, provided administrative and technical support. DR: Provided administrative and material support. The final manuscript has been approved by all authors.

TRANSPARENCY DECLARATION

The authors declare that there is no conflict of interest regarding the publication of this article.

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