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Evaluation of Extractive Values, Qualitative and Quantitative Phytochemical Constituents of Red Soko (*Celosia Trigyna*) and Green Soko (*Celosia argentea*)

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Abstract

Medicinal plants are indispensable sources of bioactive compounds and have proved to be stalwart ingredients for a wide range of applications. The potency of five different solvents in extracting bioactive constituents; qualitative and quantitative determination of phytochemicals of red soko and green soko were studied. The plant were cut into smaller pieces, air-dried, ground into powdery sample, sieved with 40 mm mesh size and properly labelled. Each sample was extracted using five different solvents (acetone, chloroform, ethyl acetate, methanol and water) at ratio 1: 10 for 72 h. Each solvent extract was screened for nine phytochemicals (flavonoid, carotenoid, phenol, oxalate, tannin, saponin, alkaloid, phytate and ascorbic acid). It was observed that the plant extract contained seven phytochemicals in both red and green soko. The highest extractive values and qualitative screening of phytochemicals in red soko and green soko were obtained in water and methanol extracts. Quantitative phytochemical analysis showed that there was higher content of saponin, phytate and ascorbic acid in the two vegetables. Red soko contained lower ascorbic acid, saponin, total phenol, total carotenoid, alkaloid and flavonoid than green soko while green soko had lower phytate and tannin than red soko. There was no significant difference ($P<0.05$) in flavonoids, total carotenoid and alkaloid contents in red soko and in green soko there was no significant difference ($P<0.05$) in total carotenoid and alkaloid contents.

Keywords

Phytochemical, solvent, extractive values, red soko and green soko

1. Introduction

Phytochemical means plant chemicals, and they are plant secondary metabolites which have little or no role in photosynthesis, respiration, or growth and development, but may accumulate in surprisingly high concentrations [1]. Phytochemicals can be defined as plant-derived chemicals, which are beneficial to human health and disease prevention [2]. They give plants its colour, flavour, smell and are part of a plant's natural defense system (disease resistance). Phytochemicals are bioactive, non-nutrient plant compounds that can be found in fruits, vegetables, grains and other plant foods that have been linked to reducing the risk of major degenerative diseases [3, 4]. Plant foods provide not only es-

sential nutrients needed to sustain life, but also afford bioactive compounds (phytochemicals) for health promotion and disease prevention [5-7]. Phytochemicals include phenolic compounds, alkaloids, nitrogen containing compounds, saponins, terpenoids, organosulfur compounds and carotenoids [7]. About 200,000 structures of phytochemicals are known and there are close to 20,000 (10%) of them that have been identified as originating from fruits, vegetables, and grains [8]. In cereals, phenolic compounds are the major phytochemical [9]. The daily requirements for bio-available micronutrients and phytochemicals are obtained through the consumption of indigenous leafy vegetables [10] which are usually abundant during the rainy season. Indigenous leafy vegetables may be a rich source of phenolic compounds and other phytochemicals that contribute to the antioxidant activity in the diet [10], thus providing strong protective effects against major diseases associated with oxidative damage [11].

Vegetables play an important role in human diets, as they support the normal functioning of the different body systems. They provide our cells with vitamins, minerals, fiber, essential oils and phytonutrients. Vegetables contain low amounts of fat and calories [12]. Leaf vegetables came from very wide variety of plants and they are plants with edible leaves. Each of us knows lettuce and spinach, as well as mustard, but also early springtime nettles are valuable source of vitamin C. Green leafy vegetables are popularly used for food, being a rich source of β-carotene, ascorbic acid, minerals and dietary fiber. One of the most popular vegetable is lettuce. Lettuce is cultivated worldwide, and is one the most consumed green leafy vegetables in the raw form for its taste and high nutritive value, being regarded as an important source of phytochemicals, including carotenoids, in the diet [13]. Vegetables are the greatest sources of phytochemicals and facts have emerged that some anti-nutritional content of these vegetables have potentials in reducing some diseases in man [13]. Some of these diseases include high blood pressure, heart attack, stroke and other cardiovascular diseases [14]. Leafy vegetables are natural source of antioxidants and rich in phytochemicals [15-16].

Celosia, Lagos spinach, is an important leaf vegetable for millions of households in sub-saharan Africa because of its multifaceted usefulness. In south-western Nigeria, it is known as “sokoyokoto” (Yoruba) [17]. The Celosia species is a genus and herbaceous of edible and ornamental plants of the family Amaranthaceae. The generic name is derived from the Greek word kelos, meaning "burned," and refers to the flame-like flower heads [18]. In Nigeria, six species of the genus Celosia have been described [19]. The leaves and stems are cooked into soups, sauces or stew with other ingredients [17]. The leaves and tender stem of plumbed cockscomb (*Celosia species*) are consumed as a vegetable and the inflorescence eaten as a herb [20-21]. Due to civilization, *Celosia* species are almost gone into extinction, therefore the focus of this research work is to determine the effectiveness of solvents in extracting the bioactive compounds in red soko and green soko as well as knowing which of the two vegetables is richer in phytochemicals with view of establishing their usefulness and gardening.

2. Materials and methods

2.1 Source of materials

The plants (green soko and red soko) were collected from a local farms in Owo, Ondo State, Nigeria. All chemicals used were of the analytical grade with the highest purity available (>99.5%) and procured from Sigma Aldrich, USA.

2.2 Preparation and extraction of red soko and green soko

The plant materials (green soko and red soko) used were rinsed in water, cut into smaller pieces for easy drying, air-dried, ground and finally sieved to give 40 mm mesh size powder. They were put in air-tight containers and kept in a refrigerator at 4^0C prior to analysis. The powdered samples were divided into portions, packed in air tight containers labelled appropriately prior to extraction. Each sample was extracted separately with each solvent (acetone, chloroform, ethyl acetate, methanol and water) at ratio 1:10 for 72 h during which it was intermittently shaken on a shaking orbit machine The resulting mixture was filtered through a 0.45 µm nylon membrane filter. The extracts were desolventised to dryness under reduced pressure at 40 ^{o}C by a rotary evaporator (BUCHI Rotavapor, Model R-124, Germany). Weight of extract obtained was used to calculate the percentage yield (extractive value) of extract in each solvent and the dry extracts were stored in a refrigerator (4 ^{0}C) prior to analysis [22]-[24].

2.3 Phytochemical analysis

Both qualitative and quantitative analyses were carried out. The presence of major phytochemical secondary metabolites, namely, saponins, alkaloids, flavonoids, tannins, phenolics, and terpenoids were determined using standard phytochemical methods with some modifications [25].

3. Qualitative determination of phytochemicals

3.1 Test for flavonoids (Cyanidine test)

This was done according to the method of Stankovic [26]. About 0.2 g of the plant sample/extract was added with 2

mL methanol and 1 mL of concentrated sulphuric acid added. A spatula was used to add a powder of magnesium chloride ($MgCl_2$) and the mixture observed for 1 min for effervescence and also observed for a brick red colouration.

3.2 Test for phenol

Small quantity of the extract/ plant sample (about 0.5 g) was added to about 0.5 mL of $FeCl_3$ solution. A deep bluish green solution was an indication for the presence of phenol [27].

3.3 Test for ascorbic acid

Plant samples/extract were crushed in acetic acid and filtered. Few drops of 2, 6-dichlorophenolindophenol solution to the 0.5ml of the filtrate. The presence of faint pink confirmed that ascorbic acid was present [28].

3.4 Test for saponin

About 0.2 g of the extract/plant sample was shaken with 5ml of distilled water and then heated to boil. Frothing (appearance of creamy miss of small bubbles) showed the presence of saponin [27].

3.5 Test for tannin

About 0.2 g of plant sample/extract was stirred with 5 ml of distilled water and later filtered. Few drops of $FeCl_3$ solution was added to 1ml of the filtrate. A blue-black green or blue green precipitate was an evidence for the presence of tannin [27].

3.6 Test for alkaloid

Test for alkaloids (Wagner's test). This was done according to the method of Joshi *et al.* [29]. About 0.2 g of the plant sample/extract was stirred with 0.4 mL of 1% HCl in a water bath for 5 min and filtered. Two grams (2 g) of Potassium iodide and 1.27 g of iodine were dissolved in 5 mL of distilled water and the solution was diluted to 100 mL with distilled water. Two drops of this iodine solution were added to the filtrate; a brown coloured precipitate indicated the presence of alkaloids. (0.5 mL) of juice was added to 2 mL of glacial acetic acid containing two drop of ferric chloride. The set up was underplayed with 1 mL of concentrated sulphuric acid. It was observed for the appearance of violet and brownish rings below the interface, followed by the formation of a greenish ring in the acetic acid layer.

3.7 Test for oxalate

About 0.5 g of sample/extract was boiled with 1 ml of 2% H_2SO_4 solution on water bath. It was filtered while warm and few drops 1% $KMNO_4$ was added. Pink colour confirms the presence of oxalate [30].

3.8 Test for phytate

About 0.5 g of the sample/extract was mixed with 2 ml of 2% HCl solution. It was filtered and two drops of 0.3% ammonium thiocynate (NH_4SCN) solution and 2 ml of distilled water were added and shaken. 3 to 4 drops of 10% $FeCl_3$ solution were then added. Yellow colouration indicates the presence of phytate [30].

3.9 Test for carotenoids

About 0.5 g of the sample/ extract was mixed with 2 ml of distilled water. 5 ml of 2%w/v alcoholic KOH solution was added and the mixture was heated on a water bath for 10 minutes. 2 ml of chloroform and 0.5 g of Na_2SO_4 were added and shaken thoroughly. A violet colour indicates the presence of carotenoids [30].

4. Quantitative determination of phytochemicals

4.1 Determination of flavonoids

AOAC [31] method was used for this analysis. 0.50 g of finely ground sample was weighed into a 100 mL beaker, 80 mL of 95% ethanol was added and stirred with a glass rod to prevent lumping. The mixture was filtered through a What-man No. 1 filter paper into a 100 mL standard flask and made up to mark with ethanol. 1ml of the extract was pipetted into 50 mL standard flask, four drops of concentrated HCl was added via a dropping pipette after which 0.50 g of magnesium turnings was added to develop a magenta red coloration. Standard flavonoid solution of range 0-20 ppm were prepared from 100 ppm stock solution and treated in a similar way with concentrated HCl and magnesium turnings as for the sample. The absorbance of magenta red coloration of sample and standard solutions were read on a digital Jenway V6300 Spectrophotometer at a wavelength of 520 nm. The flavonoid was calculated using the formula:

$$\text{Flavonoids (ppm)} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample}}$$

4.2 Determination of total carotenoids

The procedure of AMC-RSC [32] was adopted. 2 g of each sample was weighed into a flat bottom reflux; 10 mL of distilled water was added and shaken carefully to form a paste. 25 mL of alcoholic KOH solution was added and a reflux condenser attached. The above mixture was heated on a boiling water bath for 1hour during which it was carefully and frequently shaken. The mixture was cooled rapidly under tap water and 30 mL of water was added. The hydrolysate obtained was transferred into a separating funnel. The solution was re- extracted three times with 25 mL of chloroform. 2 g anhydrous Na_2SO_4 was added to the extract to remove any traces of water, the mixture was then filtered into 100 mL standard flask and made up to mark with chloroform. Standard solution of β- carotene vitamin A of range 0-50 g/mL were prepared with chloroform. The above gradients of different standard prepared were determined and the average gradient was taken to calculate vitamin A (β- carotene in µg/100g). Absorbance of sample and standard solutions were read on the spectrophotometer (Digital Spetronic 21D Spectrophotometer) at a wavelength of 329 nm.

$$\text{Carotenoid (Vitamin A) µg/100g} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample} \times 100}$$

Carotenoid (Vitamin A) ppm = Carotenoid (Vitamin A) µg/100g x 10^{-2}

4.3 Conversion

6mg of β- carotene = 1 retinol equivalent
12mg of other Biological Active Carotenoids = 1: 1 Retinol equivalent
1 retinol equivalent of Vitamin A activity = 1mg retinol
1 retinol equivalent 3.I.U.

4.4 Determination of total phenol

About 0.20 g of sample was weighed into a 50 mL beaker, 20 mL of acetone was added and homogenized properly for 1 hour to prevent lumping. The mixture was filtered through a What man No.1 filter paper into 100 mL standard flask using acetone to rinse and made up to mark with distilled water with thorough mixing. 1 mL of sample extract was pipetted into 50 mL standard flask, 20 mL water was added, 3 mL of phosphomolybdic acid was added followed by the addition of 5 mL of 23% Na_2CO_3 and mixed thoroughly. The mixture was made up to mark with distilled water and allowed to stand for 10 minutes to develop bluish green colour. Standard phenol of concentration range 0-40 mg/L was prepared from 100 mg/L stock phenol solution from Sigma Aldrish Chemicals, U.S.A. The absorbance of the sample as well as that of the standard concentration of phenol was read after 30 minutes in 1cm cell on a digital Spectrophotometer at a wavelength of 510 nm. The total phenol in ppm was calculated thus:

$$\text{Total phenol (ppm)} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample} \times 100} \quad [25]$$

4.5 Determination of oxalate

About 1 g of each sample was weighed into 250 mL conical flask and soaked with 100 mL of distilled water. They were allowed to stand for 3 hours and each was filtered through a double layer of filtered paper. Standard solution of oxalic acid range 0-40 ppm concentrations were prepared and read on a digital spectrophotometer at 420 nm in 1cm cell for absorbance. The absorbance of filtrate from each sample was also read.

$$\text{Oxalate (ppm)} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample} \times 100} \quad [25]$$

4.6 Determination of tannin

1 g of each sample was weighed into a beaker. Each was soaked with solvent mixture (80 mL of acetone and 20 mL of glacial acetic acid) for 5 hours to extract tannin. The whole mixture was filtered through a double layer filter paper to obtain the filtrate. A set of standard solution of tannic acid was prepared ranging from 10 ppm to 50 ppm. The absorbance of the standard solution as well as that of the filtrates were read in 1cm cell at 760 nm in a digital spectrophotometer.

$$\text{Tannin (ppm)} = \frac{\text{Absorbance of sample} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample} \times 100} \quad [33].$$

4.7 Determination of saponin

1 g of finely ground sample was weighed into a 250 mL beaker and 100 mL of isobutyl alcohol was added. The mixture was shaken on a UDY shaker for 5 hours to ensure uniform mixing. Thereafter the mixture was filtered through What man No 1 filter paper into a 100 mL beaker and 20 mL of 40% w/v saturated magnesium trioxocarbonate (iv) solution was added. The mixture obtained with saturated magnesium trioxocarbonate (iv) solution was again filtered through What man No.1 filter paper to obtain a clear colourless solution. 1 mL of colourless solution was pipetted into 50 mL volumetric flask and 2 mL; of 5% w/v $FeCl_3$ solution was added and made up to mark with distilled water. It was allowed to stand for 30 minutes for blood red colour to develop. 0-50 ppm standard saponin solution were prepared from 1000 ppm saponin stock solution. The standard solutions were treated similarly with 2 mL of 5% $FeCl_3$ solution as done for 1 mL sample above. The absorbance of the sample as well as standard saponin solutions were read after colour development in a Jenway V6300 Spectrophotometer at a wavelength of 350 nm.

$$\text{Saponnin (ppm)} = \frac{\text{Absorbance of sam ple} \times \text{Gradient factor} \times \text{Dilution factor}}{\text{Weight of sample} \times 100}$$ [34].

4.8 Determination of alkaloids

Gravimetric method was used for this determination. 2 g of finely ground sample was weighed into a 100 mL beaker and 50 mL of 10% acetic acid solution in ethanol was added. The mixture was shaken well and allowed to stand for 4 hours before filtering. The filtrate was evaporated to one quarter of its original volume. Concentrated NH_4OH was then added drop wise to fully precipitate the alkaloids. The precipitate was filtered off with a weighed filter paper (W_1) and washed with 1% NH_4OH solution. The precipitate in the filter paper was dried in an oven at 60 ^{0}C for 30 minutes and reweighed (W_2). The weight of alkaloid was determined by weight difference and it was expressed as ppm.

$$\text{Alkaloid (ppm)} = \frac{\text{Weight difference } (W2 - W1) \times 10^6}{\text{Weight of sample}}$$ [33, 35].

4.9 Determination of phytates

2 g of each sample was weighed into 250 mL conical flask. 100 mL of 2% v/v concentrated HCl was used to soak the sample for 3 hours in the conical flask. This was filtered through a double layer of hardened filter paper. 50 mL of filtrate was placed in 400 mL beaker and 107 mL of distilled water was added to give acidity (PH 4.5). 10 mL of 0.3% ammonium thiocyanate solution was added into the solution as indicator. This was titrated with standard $FeCl_3$ solution which contained 0.00195 g Fe per mL. The end point is slightly brownish yellow which persisted for 5 minutes. The phytate was calculated using the formula:

$$\text{Phytate (ppm)} = \frac{\text{Titre value} \times 0.00195 \times 1.19 \times 10^6}{\text{Weight of sample}}$$ [36].

4.10 Determination of vitamin C

0.05 g of 2, 6 – dichlophenol lindophenol was dissolved in100 mL of distilled water and filtered. To standardize, 0.05 g of pure ascorbic acid was dissolved in 60 mL of 20% glacial acetic acid and the solution was made up to exactly 250 mL with distilled water. 10 mL of this solution was pipetted into a small flask and titrated against the indophenol solution until a faint pink colour was obtained. Colour persisted for 15 seconds and volume of indophenol (V_{mL}) of dye equals 0.05 g ascorbic acid.

5 g of the sample was mixed with 100 mL of distilled water and filtered, 10 mL of the filterate was taken into 100 mL standard flask , 20 mL of 20% glacial acetic acid was added and the flask was made up to mark with distilled water. 10 mL of the resultant solution was pipetted into a conical flask and titrated with the standard indophenol solution (Y). The ascorbic acid (Vitamin C) was calculated as:

$$\text{Vitamin C (ppm)} = \frac{\text{Y} \times 0.05 \times 10 \times 10^6}{\text{Weight of sample } (W) \times \text{Volume (V)}}$$ [37]

4.11 Statistical Analysis

Statistical significance tests were performed using SPSS (v. 20, IBM SPSS Statistics, US) at $p < 0.05$ by means of one-way analysis of variance (ANOVA) followed by LSD post hoc multiple comparison and the experimental results were expressed as mean ± standard mean deviation of three replicates.

4.12 Results and discussion

The extractive values (% yield) of red soko and green soko in different solvents is presented in Table 1. The percentage yield of red soko extract in acetone, chloroform, ethylacetate, methanol and water were 0.94±0.01%, 1.98±0.03%,

0.58±0.00%, 5.50±0.09% and 5.91±0.11% accordingly. There was no significant difference (P<0.05) in extractive values of acetone and ethylacetate red soko extracts. Methanol and water extracts of red soko had no significant difference (P<0.05) in extractive values. The extractive value of red soko was highest in water, followed by methanol, chloroform, acetone ad least in ethylacetate.

For green soko, the extractive values in acetone, chloroform, ethylacetate, methanol ad water were 0.39±0.00%, 1.50±0.01%, 1.49±0.03%, 5.39±0.11% and 7.81±0.14% respectively. There was no significant difference (P<0.05) in extractive values of methanol and water green soko extracts. There existed no significant difference (P<0.05) in extra c-tive values of chloroform and ethylacetate extracts of green soko. Acetone had the lowest extractive value in green soko while water had the highest extractive value. In all the five solvents used for extraction, the extractive values in water and ethylacetate were higher in green soko than red soko. While the extractive values in red soko was higher in acetone, chloroform and methanol than green soko. Generally, there are quite number of factors in which extraction of bioactive compounds depends. The selection of solvent system largely depends on the specific nature of the compounds being targeted [38].

Table 1. Extractive values (% yield) of red soko and green soko in different solvents

Samples	Acetone	Chloroform	Ethylacetate	Methanol	Water
Red soko (%)	0.94^{a}±0.01	1.98^{b}±0.03	0.58^{a}±0.00	5.50^{c}±0.09	5.91^{c}±0.11
Green soko (%)	0.39^{a}±0.00	1.50^{b}±0.01	1.49^{b}±0.03	5.39^{c}±0.11	7.81^{c}±0.14

NOTE: Within each row, mean values followed by the same superscript are not significantly different at P<0.05 level according to Duncan's New Multiple Range Test (DMRT); Values represent means of triplicate determination ±standard deviation.

Table 2. Qualitative phytochemical screening of raw red soko and its solvents extracts

Parameters	Raw red soko	Solvent extracts				
		Acetone	Chloroform	Ethyl acetate	Methanol	Water
Flavonoid	+	–	–	–	–	–
Carotenoid	-	–	–	–	–	–
Phenol	+	–	–	–	–	–
Oxalate	+	–	–	–	+	+
Tannin	+	–	–	–	–	+
Saponin	+	–	–	–	+	+
Alkaloids	-	–	–	–	–	–
Phytate	+	–	–	–	+	+
Ascorbic Acid	+	–	–	–	+	+
% Phytochemical detectable	77.8	0	0	0	44.5	55.5

+ =Present - = Absent

Table 2 depicts qualitative phytochemical of screening of raw red soko and its solvents extract of red soko. Flavonoids, carotenoids, phenol, oxalate, tannin, saponin, alkaloids, phytate and ascorbic acid were the nine (9) phytochemicals screened for in the red soko. Seven (7) phytochemicals were present in red soko. Carotenoids and alkaloids were absent in the red soko and this made the percentage phytochemical detectable in red soko was 77.8%. The solvents used for extraction were acetone, chloroform, ethylacetate, methanol and water. It was obvious that there was no phytochemical present in solvent extract of acetone, chloloroform, ethylacetate in red soko. Only oxalate, saponin, phytate and ascorbic acid were present in methanol extract of red soko, and this gave 44.5% phytochemical extractable by methanol. There were presence of oxalate, tannin, saponin, phytate and ascorbic acid in water extracts of red soko and the percentage phytochemical detected in water extract of red soko was 55.5%. The high extractive propensity for bioactive ingredients in red soko by methanol and water may be attributed to high and strong polarity that existed in the two solvents.

The qualitative phytochemical screening of raw green soko and its solvent extracts is shown in Table 3. Flavonoids, carotenoids, phenol, oxalate, tannin, saponin, alkaloids, phytate and ascorbic acid were the nine (9) phytochemicals screened for in the green soko. Seven (7) phytochemicals were present in green soko. Oxalate and alkaloids were absent in the green soko and this made the percentage phytochemical detectable in green soko was 77.8%. Ethylacetate extract

of green soko contained only saponin which gave 11.1% phytochemical detectable. Methanol extract of green soko contained phenol, saponin and ascorbic acid amounting to 33.3% phytochemical detectable. Water extract of green soko showed presence of carotenoid, phenol, tannin, saponin, phytate and ascorbic acid which amount to 66.7% of phytochemical detectable. None of the phytochemical were found present in acetone and chloroform extracts of green soko. Water had highest extractable phytochemicals in green soko and this was followed by methanol.

Table 3. Qualitative phytochemical screening of raw green soko and its solvent extracts

Parameters	Raw green soko	Solvents extracts				
		Acetone	Chloroform	Ethylacetate	Methanol	Water
Flavonoid	+	–	–	–	–	–
Carotenoid	+	–	–	–	–	+
Phenol	+	–	–	–	+	+
Oxalate	-	–	–	–	–	–
Tannin	+	–	–	–	–	+
Saponin	+	–	–	+	+	+
Alkaloids	-	–	–	–	–	–
Phytate	+	–	–	–	–	+
Ascorbic Acid	+	–	–	–	+	+
%Phytochemical detectable	77.8	0	0	11.1	33.3	66.7

+ =Present - = Absent

Table 4. Quantitative phytochemical analysis of red soko and green soko

Samples	Red Soko	Green Soko
Flavonoids (ppm)	8.02^{a}±0.06	9.50^{ab}±0.09
Total Carotenoids(ppm)	6.51^{a}±0.04	7.47^{a}±0.06
Total Phenol (ppm)	16.00^{b}±0.32	20.00^{c}±0.54
Oxalate (ppm)	21.79^{bc}±0.57	12.01^{b}±0.34
Tannin (ppm)	15.03^{b}±0.48	13.10^{b}±0.41
Saponin (ppm)	30.98^{c}±0.34	38.51^{e}±0.55
Alkaloids (ppm)	4.58^{a}±0.03	5.49^{a}±0.02
Phytate (ppm)	232.00^{d}±1.20	116.13^{f}±1.11
Ascorbic acid (ppm)	25.12^{bc}±0.37	28.95^{d}±0.33

NOTE: Within each column, mean values followed by the same superscript are not significantly different at P≤0.05 level accord ing to Duncan's New Multiple Range Test (DMRT); Values represent means of triplicate determination ±standard deviation

Table 4 depicts the quantitative phytochemical analysis of red soko and green soko. The phytochemicals quantified in the two leafy vegetables (red soko and green soko) were flavonoids, total carotenoids, total phenol, oxalate, tannin, saponin, alkaloids, phytate and ascorbic acids. The flavonoid content of red soko was 8.02±0.06 ppm and green soko was 9.50±0.09 ppm. Flavonoid are known to have primary antioxidant activity and free radicals scavengers [39]. They also show multiple biological activities, they are antibacterial, anti-inflammatory, carcinogenic, anti-allergic, antiviral [40]. The total carotenoids of red soko and green soko were 6.51±0.04 ppm and 7.47±0.06 ppm respectively. The total carotenoid was lesser than flavonoids in the two samples. Carotenoids are the pigments found in yellow-orange vegetables and leafy green vegetables, it is known to enhance immune functions. Many of the carotenoids are antioxidant that protect cells against free radicals by neutralizing than before they cause oxidative damage [41]. The total phenol was higher in green soko (20.00±0.54 ppm) than red soko (16.00±0.32 ppm). Phenol had antioxidant capacities that are much stronger than those of vitamin A and E [42]. Phenolic phytochemical inhibit autoxidation of unsaturated lipids, thus preventing the formation of oxidixed low-density liproprotein (LDL) which is considered to induce cardiovascular dis-

ease [43]. Green soko had the lower oxalate content (12.01±0.34 ppm) than red soko (21.79±0.57 ppm). Oxalate is produced and accumulated in many crop plants and pasture weed. It may be present in plants as the soluble salt of potassium, sodium or ammonium oxalate as oxalic acid or as insoluble calcium oxalate [44]. The tannin content in red soko and green soko were 15.03±0.48 ppm and 13.1±0.41 ppm. It was reported the plant tannins are source of some commercial tannic acid as tannin agent [45] and they have ability to inhibit HIV replication selectively and is also used as diuretics [45]. Red soko had the lower saponin value (30.98±0.38 ppm) than green soko (38.51±0.55 ppm). Saponin is a group of glycosides widely distributed in plants, which forms durable foam when their watery solutions are shaken which even in high dilutions dissolve erythrocyte. Medically, it has been reported that saponin is used in hypercholesterolaemia, hyperglycaemia, antioxidant, anti-cancer, anti- inflammatory and weight loss, etc, [46]. Saponin also has anti carcinogenic agents, immune modulations activity, cholesterol lowering activity and anti-fungal properties [47]. The alkaloid in red soko was lower (4.58±0.03 ppm) than green soko (5.49±0.02 ppm). Plants contain alkaloids have been reported to be effective in medicinal plant that are capable of reducing headaches associated with hypertension [48]. And they are also used in the management of cold, chronic catarrh, persistent headaches and migraine [49] and eating plants with high concentration of alkaloids has been reported to be toxic because it interferes with the digestive process [50] and thereby inhibits the efficient utilization of nutrie6nts [51]. Red soko had the higher value of phytate (232.00±1.20 ppm) than green soko (116.13±1.11ppm). Phytate has a strong binding affinity to important minerals such as calcium, magnesium, iron and zinc and as such it forms insoluble precipitate and will be non-absorbable in the intestines. It has therapeutic uses as phytonutrient and also provides antioxidant effect. Phytate also has mineral binding properties making it to prevent colon cancer by reducing oxidative stress in the line of the intestinal tract [44]. The ascorbic acid content of red and green soko were 25.12±0.37 ppm and 28.95±0.33 ppm respectively. Green soko contained higher content of vitamin C than red soko showed. Ascorbic acid helps in absorption of iron in the gut; to form collagen in bones, cartillages and muscles [52]. Vitamin C is an antioxidant that facilitate the transport and uptake of non-heme iron at the mucosa, the reduction of folic acid intermediates and the synthesis of cortisol. The deficiency are scurvy, decay of gum and fragility to blood capillaries [53, 54]. Ascorbic acids (vitamin C) is an anticancerous agent that helps to fight and guide the body against cancer and other degenerative diseases such as Type 2 diabetes mellitus and arthritis [55]. There was significant different ($p< 0.05$) between the two species of leafy vegetables of flavonoid values. There was no significant difference ($p<0.05$) in flavonoids, total carotenoids and alkaloids; total phenol and tannin; and oxalate and ascorbic acid in red soko and there was no significant difference ($p<0.05$) in total carotenoid and alkaloids; and oxalate and tannin in green soko.

5. Conclusion

There was slight difference in phytochemical profile of red soko and green soko and this was also dependent on the nature of the solvents used for extraction. Methanol and water had the highest extractive values for red soko and green soko. Both vegetables were very rich in ascorbic acid, phytate and saponin but green soko was richer in ascorbic acid, saponin, total phenol, total carotenoid, alkaloid and flavonoid than red soko. Red soko had higher phytate and tannin than green soko. Nutritionally, green soko is slightly richer in phytochemicals than red soko.

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