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Evaluation of free radical scavenging capability of *Telfairia occidentalis* (UGU) and *Pterocarpus soyavxii* (OHA) ethanolic leaf extracts

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Abstract
Vegetables are of interest to food scientist and fitness-conscious individuals for management of stress and human ailments. In this study we compared the antioxidant capabilities of ethanol extracts of *Telfairia occidentalis* (Ugu) And *Pterocarpus soyavxii* (Oha) by assessing their abilities to scavenge superoxide anion (O_2.-), 2,2-diphenyl-1-picrylhydrazyl (DPPH), and nitric oxide radicals (NO.) using appropriate assay systems. From the results Ugu extract showed a better superoxide radical inhibitory capability relative to Oha. The IC_{50} for $O_2^{\cdot-}$ anion inhibition was significantly lower ($p<0.05$) for Ugu extract 52.76 ± 4.44 µg/ml compared to Oha extract 524.04 ± 11.58 µg/ml. Ugu extract inhibited 88.69 ± 3.11 % of DPPH· at a concentration of 1000 µg/ml compared to Oha extract that inhibited 69.62 ± 4.77 % and ascorbic acid which inhibited 96.41 ± 1.92 % at same concentration of 1000 µg/ml. The extracts exhibited strong NO· radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The observed free radical inhibitory action of these vegetables suggests that they could be of great relevance in the food industry, as well as a source of dietary antioxidant, reducing lipid oxidation in the food products in addition to managing diseases whose pathogenesis implicate oxidative stress.

Keywords: *Telfairia occidentalis*, *Pterocarpus soyavxii*, free radical, antioxidant

1. Introduction
Vegetables are low in fat but contain good amount of vitamins and minerals. All the green-yellow-orange vegetables are rich source of calcium, magnesium, potassium, iron, beta-carotene, vitamin B-complex, vitamin C, vitamin A, and vitamin K. Vegetables are home for many antioxidants that help protect the human body from oxidant stress, diseases and cancer, and also help the body develop the capacity to fight against these by boosting its immunity [1].

In order to improve immune system, vitamins, minerals and antioxidants are essential. Most vegetables are very rich in phytonutrients and a host of antioxidants which assure a normal progression of the metabolic processes of the body. The nutrient composition of different type of vegetables varies considerably and contains vitamins, essential amino acids as well as minerals and oxidants that play several roles in the body [2].

Two varieties of vegetable (*Telfairia occidentalis and Pterocarpus soyavxii)* were evaluated in this research for antioxidant and oxidant levels using standard analytical technique. Recently antioxidants have been used to prevent the formation of free radical caused by oxidants. *Telfairia occidentalis* commonly called ugu leaf in igbo language is very low in Cholesterol, it is also a good source of Calcium, and a very good source of Protein, Vitamin A, Vitamin C, Thiamin, Riboflavin, Niacin, Vitamin B6, Folate, Iron, Magnesium, Phosphorus, Potassium, Copper and Manganese. *Pterocarpus soyavxii* commonly called oha leaf has a low content of anthocyanin and carotenoids while alkaloid was most abundant in it [3].

2. Materials and Methods
2.1 Chemicals
All the chemicals used in this study were of analytical grade. The solvent ethanol, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical, Methionine, Riboflavin, Ethylene diamine tetra acetic acid (EDTA), Nitro blue tetrazolium (NBT), Phosphate buffer, Sodium chloride (NaCl), Disodium hydrogen phosphate, Sodium nitro prusside (SNP), Sulfanilamide, N-naphthyl ethylene diamine, Phosphoric acid, Sodium nitride, Potassium buffer, Potassium dihydrogen phosphate (KH¬2PO4) were all purchased from Sigma Chemical Co.

2.2 Equipment
All the equipment used in this research experiment was available at the Department of Biochemistry Madonna University, Nigeria. The equipment used during this research was an Agilent 8453E UV-visible spectrophotometer for reading the absorbance of each sample mixture and an oven for drying the test tubes before each assay.

2.3 Methodology
All the plant materials (ugu and oha leaf) were purchased in Madonna University, air-dried at room temperature and reduced to fine powder by milling. The powdered plant materials were subjected to extraction with 80% ethanol. The hydroethanolic extracts were concentrated in a water bath at 45°C and stored in the refrigerator until use.

2.4 Serial Dilution
Serial dilution was done for each leaf consisting of 10 test

tubes including a blank and control for various radical scavenging assays.

Procedure for serial dilution used in each test

1. 0.1g of the extracts were measured
2. 80% ethanol was prepared from combining 20 ml of water into 80 ml of ethanol.
3. 10ml of ethanol was used to dissolve the extracts
4. Test tubes were labeled from 1 – 10 and then control and blank.
5. 9.0 ml of ethanol was pipetted to 1ml of the extracts in each test tube except the test tube labeled 1.
6. 2.0 ml of the extract in test tube 1 was extracted and imputed into test tube 2 which is 50% of the extract.
7. 2.0 ml of the extracts in test tube 2 is extracted and imputed into test tube 3.
8. 2.0 ml of the extracts from test tube 3 is extracted and imputed in test tube 4.
9. Extraction of extracts sample from one test tube to the next continues until test tube 10 where 2.0 ml is extracted and discarded.
10. For the test tube labeled control and blank had 2.0 ml of ethanol.
11. The test tube labeled control had the 2.0 ml of ethanol and 1.0 ml of DPPH.
12. The test tube labeled blank had 1.0ml of ethanol and 2.0 ml of the leaf extract

2.5 Antioxidant Assays

DPPH radical - scavenging assay

Scavenging activity on DPPH free radicals by the extract was assessed according to the method reported by Gyamfi *et al.* (1999) [4] with slight modifications [5]. Briefly, a 2.0 ml solution of the extract at different concentrations diluted two-fold (2–250 µg/ml) in ethanol was mixed with 1.0 ml of 0.3 mM DPPH in methanol. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Blank solutions were prepared with each test sample solution (2.0 ml) and 1.0 ml of ethanol while the negative control was 1.0 ml of 0.3 mM DPPH solution plus 2.0 ml of ethanol. Thereafter, the absorbance of the assay mixture was measured at 518 nm against each blank with an Agilent 8453E UV-visible spectrophotometer. Lower absorbance of the reaction mixture indicated higher radical scavenging activity. DPPH radical scavenging activity was calculated using the equation:

$$\%\ Inhibition = 100\ \% \times \left(\frac{A_0 - A_s}{A_0}\right)$$

Where A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample. The IC_{50} value represented the concentration of the extract that caused 50 % inhibition of DPPH radical and was calculated by linear regression of plots, where the abscissa represented the concentration of tested sample and the ordinate the average percent of inhibitory activity from three replicates.

2.6 Superoxide anion inhibition

This assay was based on the capacity of the extract to inhibit the photochemical reduction of nitro blue tetrazolium (NBT) [6]. The principle of reaction is similar to the DPPH radical scavenging assay as it is also quantitative. Superoxide anion is blue in colour therefore when the solution is exposed to the fluorescent light if the solution has antioxidant abilities; the blue colour formation is inhibited. Briefly, each 3.0 ml reaction mixture contained 0.05 M (1 ml) phosphate buffered saline (PBS) (pH 7.8), 13 mM (390 µl) methionine, 2 µM (60 µl) riboflavin, 100 µM (300 µl) EDTA, 75 µM (200 µl) NBT and 1.0 ml of test sample solutions (10–250 µg/ml). The tubes were kept in front of a fluorescent light (725 lumens, 34 watts) and absorbance was read at 518 nm after 20 minutes. The entire reaction assembly was enclosed in a box lined with aluminum foil. Identical tubes containing reaction mixtures were kept in the dark and served as blanks. The percentage inhibition of superoxide generation was estimated by comparing the absorbance of the control and those of the reaction mixture containing test sample as per the equation:

$$\%\ Inhibition = 100\ \% \times \left(\frac{A_0 - A_s}{A_0}\right)$$

Where A_0 is the absorbance of the control, and A_s is the absorbance of the tested sample.

2.7 Nitric oxide radical scavenging assay

Nitric oxide ($NO^{\cdot}$) generated from sodium nitroprusside (SNP) was measured according to the method of Marcocci *et al.* (1994) [7] and [5]. Briefly, the reaction mixture (5.0 ml) containing SNP (5 mM) in phosphate buffered saline (pH 7.3), with or without the plant extract at different concentrations, was incubated at 25 ºC for 180 min in front of a visible polychromatic light source (25 W tungsten lamp). The $NO^{\cdot}$ radical thus generated interacted with oxygen to produce the nitrite ion (NO_2^-) which was assayed at 30 min intervals by mixing 1.0 ml of incubation mixture with an equal amount of Griess reagent (1% sulphanilamide in 5% phosphoric acid and 0.1% naphthylethylenediaminedihydrochloride). The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulphanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was measured at 546 nm. The nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out at least three times and the data presented as an average of three independent determinations.

3. Result

3.1 Inhibitory Effect of Ugu and Oha extracts on Superoxide ($O_2^{\cdot-}$) Anion Radicals

The extracts inhibited the formation of superoxide anion via reduced NBT in a dose-related manner. As shown in Table 1, Ugu extract showed a better superoxide radical inhibitory capability relative to Oha. The maximal $O_2^{\cdot-}$ anion inhibitory activity for Ugu was 93.30 ±4.77 % at the concentration of 500 µg/ml while Oha inhibited 52.39± 4.77 % as same concentration compared to quercetin (90.47± 5.42 %). The IC_{50} for $O_2^{\cdot-}$ anion inhibition was significantly lower ($p<0.05$) for Ugu extract 52.76± 4.44µg/ml compared to Oha extract 524.04 ± 11.58µg/ml (Table 1)

Table 1: Superoxide anion radical ($o_2^{\cdot-}$) inhibition by extracts

	Superoxide AnionInhibition (%)		
Concentration (µg/mL)	**Ugu Extract**	**Oha Extract**	**Quercetin Standard**
500	93.30 ± 4.77	52.39 ± 4.77	90.47±5.42
250	85.49 ± 8.12	39.71 ± 2.32	88.09±1.31
125	77.78 ± 4.44	33.46 ± 2.47	80.54±4.76
62.5	66.83± 5.42	22.06 ± 4.04	78.57±10.48
31.25	43.63 ±4.54	19.30± 2.62	77.24±5.61
15.62	38.73±4.58	13.11 ±1.71	54.76±6.55
7.81	22.47±3.71	8.42±1.21	37.83±4.19
3.91	13.19±2.06	5.41±0.16	27.77±9.17
1.95	3.92 ± 0.95	2.92 ± 0.95	23.81±8.04
IC_{50}(µg/mL)	52.76±4.44	524.04 ±11.58	17.42±2.11

Data represented as mean ±SEM (n = 2)

3.2 Inhibitory Effect of Ugu and Oha extracts on DPPH·Radicals

The extracts showed significant dose-dependent DPPH radical scavenging capacity as shown in Table 2. Ugu extract was more efficient in inhibiting DPPH· radical compared to Oha. Ugu extract inhibited 88.69 ± 3.11% of DPPH·at a concentration of 1000 µg/ml compared toOha extract that inhibited 69.62 ± 4.77 % and ascorbic acid which inhibited 96.41 ± 1.92 % at same concentration of 1000 µg/ml. The IC_{50} values for DPPH radical inhibition for the Ugu extract was significantly lower ($p<0.05$) compared to that of Oha extract (Table 2).

Table 2: DPPH radical scavenging activity of extracts

	DPPH· Inhibition (%)		
Concentration (µg/mL)	**Ugu Extract**	**Oha Extract**	**Ascorbate Standard**
1000	88.69 ± 3.11	69.62 ± 4.77	96.41±1.92
500	70.23 ± 2.82	61.45 ± 3.24	95.09±1.37
250	65.46 ± 2.55	60.13 ± 1.32	94.75±3.03
125	56.22 ± 3.17	47.46 ± 2.47	92.43±5.45
62.5	39.52± 2.12	32.30 ± 4.04	90.95±6.74
31.25	32.78 ±3.17	19.30± 2.62	82.10±2.35
15.62	31.74±2.84	16.11 ± 1.64	74.91±1.95
7.81	22.47 ±2.85	13.54± 1.41	66.07±3.96
3.91	16.91±1.43	11.19± 0.16	53.70±1.53
1.95	9.42 ± 0.63	4.92 ± 0.95	22.18±2.77
IC_{50}(µg/mL)	76.54± 4.44	128.84 ±9.58	2.42± 0.11

Data represented as mean ±SEM (n = 2)

3.3 Inhibitory Effect of Ugu and Oha extracts on Nitric Oxide ($NO^{\cdot}$) Radical

Nitric oxide ($NO^{\cdot}$) released from sodium nitroprusside (SNP) has a strong NO^{+} character which can alter the structure and function of many cellular components. This study showed that the extracts in SNP solution decreased levels of nitrite, a stable oxidation product of NO· liberated from SNP (Fig.1). The extracts exhibited strong NO· radical scavenging activity leading to the reduction of the nitrite concentration in the assay medium, a possible protective effect against oxidative damage. The NO scavenging capacity was concentration dependent with 1000 µg/ml of the extracts scavenging most efficiently compared to α-tocopherol (fig 1)

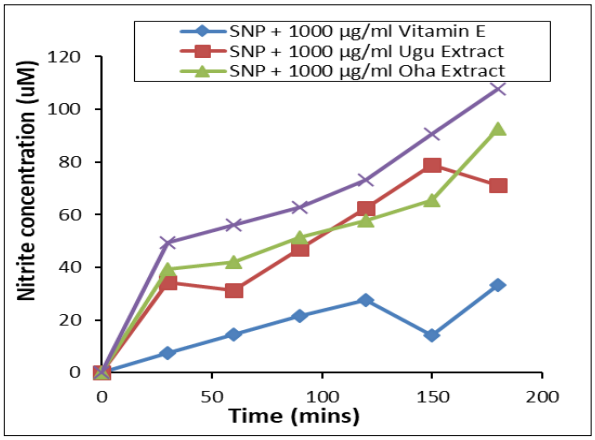

Fig 1: Effect of extract on the accumulation of nitrite upon decomposition of SNP (5 mM) at 25 ºC. Each plot represents the mean ± SEM (n = 2)

4. Discussion

Vegetable is the most widely used spice in the world today especially now people are tracing their way back to natural source of medication. Most vegetables have been shown to have anti-oxidant capability [8]. Vegetable contains an impressive list of plant derived chemical compound that are known to have disease preventing and health promoting properties. Vegetable has been in used since ancient times. Vegetable is one of the most versatile spices used virtually in all kinds of savoury cooking. In order to keep them from spoiling they are preserved in the refrigerator [9].

In this study we compared the antioxidant levels of ethanol extract of *Telfairia occidentalis* (Ugu) and *Pterocarpus soyavxii* (Oha) by assessing their abilities to scavenge superoxide ($O_2^{\cdot-}$) anion radicals, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), and nitric oxide radicals (NO.) using appropriate assay systems.

The antioxidant levels of Ugu and Oha was investigated using superoxide ($O_2^{\cdot-}$) anion radical. Statistically, Ugu showed a significantly ($p<0.05$) high mean value ($93.30 \pm 4.77\%$) of superoxide anion inhibition when compared with Oha ($52.39 \pm 4.77\%$). This shows that Ugu have a higher superoxide anion inhibitory activity at 500µg/ml concentration. Although there was no significant difference ($p<0.05$) between Ugu ($93.30 \pm 4.77\%$) when compared with the standard quercetin ($90.47 \pm 0.95\%$). Similarly, Ugu also showed a higher significant difference ($p < 0.05$) when compared to Oha at different concentration. Although Ugu at concentration 1.95µg/ml showed no significant difference ($p<0.05$) when compared with Oha ($2.92 \pm 0.95\%$). Quercetin, when compared with Oha extract showed a higher significant difference in all the concentration but showed no significant difference when compared with Ugu extract at all concentration except in concentration 1.95µg/ml where quercetin shows a higher significant difference (23.81 ± 8.04) when compared with Ugu extract (3.92 ± 0.95). This was in tandem with the work of Oboh *et al*., 2006 [10] who showed that ethanolic extract of *Telfairia occidentalis* showed high antioxidant ability.

DPPH radical is the stable free radical. It is commonly associated with the use of DPPH method to search the invitro antioxidant of pure compounds as well as plants extracts. Using DPPH, Ugu extract inhibited $88.69 \pm 3.11\%$ of DPPH at a concentration of 1000µg/ml compared to Oha extract that inhibited $69.62 \pm 4.77\%$ and ascorbate standard which inhibited $96.41 \pm 1.92\%$ at same concentration. DPPH radical inhibition for the Ugu extract was significantly lower ($p<0.05$) compared to that of Oha extract. The two vegetable extract showed good concentration dependent DPPH radical scavenging capability. Among them, Ugu leaf extract was more efficient in inhibiting DPPH radical scavenger.

In nitric oxide scavenging assay the Ugu and Oha leaf extract scavenged nitrate oxide in a time dependent manner. The nitrate oxide levels were higher in SNP sample only when compared with SNP and extract mediums at the different time interval. This suggests that SNP generated nitrate oxide but the extract with its potent nitric oxide scavenging activity, was able to mop up radicals. The possible mechanism behind these high antioxidant ability or high scavenging ability is mainly due to the presence of high secondary metabolites like phenols and saponin [11].

5. Conclusion

Since the analysis of the extract has shown the presence of some active component of the plant extract and the particular phytochemical compounds that have antioxidant activities. Further studies should help to determine the in vivo anti-oxidant activity mechanism through which these antioxidant present in this plant extract can scavenge free radical in the body against oxidative damage which can lead to serious disease. Other plants should be under studied.

6. References

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