

Assessment of antioxidant capacities and phenolic contents of nigerian cultivars of onions (*allium cepa l*) and garlic (*allium sativum l*)

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Abstract: This report assessed and compared the antioxidant potentials, quantities of ascorbic acid and phenolic compounds in methanolic extract of varieties of onions and garlic cultivars in Nigeria. The pH and total acidity of the extracts were equally determined. Antioxidancy of the cultivars were analysed using the in vitro assay techniques with 2,2-diphenyl-1-picryl Hydrazyl (DPPH) free radical scavenging and ferric reducing capacity. Ascorbic acid phenolic content were determined by volumetric and Folin-Ciocalteu's method respectively. The pH and total acidity were respectively 5.65 and 0.150mmol/L (red onion), 5.69 and 0.123mmol/L (white onion) and 6.94 and 0.105mmol/L (garlic). Red onion had the highest value of total phenols, ascorbic acid and free radical scavenging activity of 14.25±0.35mg GAE/ml, 229.098mg/100g, 66.44% respectively. In DPPH assay, red and white onion showed higher tendency to inhibit auto-oxidation when compared to garlic. The ferric reducing ability was greatest in garlic and least in white onions. These data indicate that with respect to antioxidant activity, red onion variety has highest health promoting potential among others

Keywords: Antioxidants; phenols; *allium sativum*; onions; free radical.

INTRODUCTION

Onion and garlic are vegetable species consumed world wide in large quantities. They have high flavonoid component (mainly quercetin and its conjugates) and sulphur compounds which possess antioxidant capacity (Griffiths *et al*, 2002). Alliums are known to exhibit antibacterial and antifungal properties and contain powerful antioxidants, sulphur and other numerous phenolic compounds (Noureddine, 2005). The Allium family consists of more than 700 members and each one has different taste, form and colour but are related in biochemical, phytochemical and nutraceutical contents (Tepe *et al*, 2005). Red and white *Allium cepa L* and *Allium sativum* are important part of diet in many nations due to the age long belief in their health benefits. Different types of phenolic compounds are found in allium species. These compounds are in classes of phenolic acids, flavonoids and anthocyanins. The major part of phenolics in onions present as flavonoids such as quercetin (Rivlin, 2002), isorhamnetin and kaempferol and their conjugate (Drozd *et al*, 2011). In addition, onions contain hydroxyl benzoic acids, protocatechuic acid, phloroglucinol acid and pyrocatechol (Price and Rhodes 1997). Anthocyanins such as peonidin glycosides, cyanidine glycosides and pelargonidin glycosides and their derivatives as well as delphinidin and petunidin derivatives have been identified in allium species (Naczek and Shahidi 2006).

Research reports show that onion extracts has ability to prevent cardiovascular diseases because it is capable of

reduce bad cholesterol, bad lipids, hypertension, diabetes and other similar diseases (Faller and Fialho, 2009; Gorinstein *et al*, 2009). The antioxidant activity of onions and garlic have been studied in several models and assays (Shon, *et al* 2004; Takahashi and Shibamoto, 2008; Singh *et al*, 2009; Siti *et al*, 2011; Irda *et al*, 2013; Heidarpour *et al*, 2013). The health properties *allium* vegetables have been highlighted in numerous in vitro, in vivo, and ex-vivo studies (Tuyns *et al*, 1988; Dorant *et al*, 1996; El-Demerdash *et al*, 2005; Galeone *et al*, 2006, Lawson, *et al*, 1998).

The various analytical methods of evaluation of the antioxidant capacity fall into distinct categories of electrochemical and spectrometric techniques. Hence, Cyclic Voltammetry (Borowski *et al* 2008), Amperometry (Chong and Olsher, 2007) and various spectrometric methods such as DPPH (2,2-diphenyl-1-picrylhydrazyl) method (Pisoschi *et al*, 2009), ABTS (2,2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonate) method (Marcet *et al*, 2004), FRAP (ferric reducing antioxidant power) method (Gil *et al*, 2002), The ORAC (oxygen radical absorption capacity) assay (Denev *et al*, 2010), HORAC (Hydroxyl Radical Antioxidant Capacity method (Ou *et al*, 2001), PFRAP (Potassium Ferricyanide Reducing) Fluorimetry (Jayaprakasha *et al*, 2008) have been variously used to determine the antioxidant capacities of many vegetables and plants.

Interest in natural antioxidants is increasing because synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are

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suspected to be carcinogenic (Lawson *et al* 2008; Velioglu *et al* 1988) and pathogenesis of most diseases (Olajire and Azeez, 2011).

Nigeria cultivars of onions and garlic are grown during wet and dry seasons around the savannah ecological zone and on well-drained medium textured soils with pH 6.0-7.0 for optimum yield. The temperature requirement is between 15°C to 25°C, low atmospheric humidity and clear bright days. Onions grown in Nigeria are often referred to as short day cultivars (Kudi *et al*, 2008).

This present work estimated the antioxidant capacity ascorbic acid and total phenolic content in Red Onions, White Onions (*Allium cepa* L) and Garlic (*Allium sativum*).

MATERIALS AND METHODS

Equipment

The following equipment were utilised in the experimentation. Water bath (electric thermo stated; Labnet 6 litres); Rotary evaporator (Jenway RE300); Weighing balance (B. Bran L P 203 Electronic Balance); pH Meter (Mettler Toledo) and UV-Visible Spectrophotometer (Ultrospec 4000 Pharmacia Biotech, Cambridge).

Samples and samples preparation

Samples of garlic (*A. sativum* L) and onion (red and white) (*A. cepa*) were purchased from the local market in Awka, Anambra State, Nigeria. The samples were randomly selected off the shelf based on their freshness. The samples were confirmed by the taxonomy unit of the Department of Plant Science and Biotechnology of the University of Nigeria, Nsukka, Nigeria. The samples were cut into smaller pieces to ease the drying process. The drying process was done at 60°C and 70°C in a hot circulatory oven and then ground into powder using a manual grinder. The powdered mass obtained was stored in an airtight container for further analysis.

Extraction

The extraction was done as reported in earlier work by Ifesan and co-worker (2014). Methanol: water mixture (70:30 v/v) was added to the pulverized material and continuously stirred for 24 h. Each extract was filtered through four-layer cheesecloth and the process repeated until all soluble compound had been extracted. Extraction was considered to be complete when the filtrate had a faint colour. After the extraction process, the samples in the round bottom flask were subjected to rotary evaporation to remove the extracting solvent from the extracts. Finally, the extracts were subjected to freeze drying to remove water from the extracts. The extracts were kept in the refrigerator for further use.

Preparation of reagents

Standardisation of 0.1M NaOH using potassium hydrogen phthalate, preparations of 4% Oxalic Acid Solution; 0.001M 2, 6 Dichlorophenol Indophenol Dye Solution; 10 Fold Dilution Of Folin Ciocalteu's Reagents (i.e 1:10); 2% Na₂CO₃ Solution; 1% Potassium Ferricyanide Solution; 10% Trichloroacetic Acid Solution; 0.1% Ferric Chloride Solution were all prepared according to well known classical method in Chemistry.

Estimation of acidity and pH

The samples total acidity were estimated using titrimetry. In this method 10% of the samples were prepared by dissolving 10g of the sample in distilled water and made up to mark in 100cm³ volumetric flask and 10cm³ of the sample solution was transferred into a conical flask. To 10cm³ of the sample solution, 2 drops of methyl orange and 4 drops of phenolphthalein were added. After mixing, the solution was titrated with 0.1M NaOH until a salmon pink colour was obtained. The volume of NaOH used up was recorded. Then the titration was continued to a pink coloured endpoint and the volume of NaOH used was recorded. The total acidity was obtained using the formulae.

$$\text{Total acidity} = [T \times M \times 0.75] / [V \times 10 \times 0.1]$$

where T is the volume of 0.1M NaOH, M is the molarity of approximate 0.1M, V is the volume of the sample and is expressed in mmol/l.

The pH of samples solutions were estimated using pH meter.

Ascorbic acid assay

This was done volumetrically as described in literature (Li *et al*, 2007). Ascorbic acid solution (0.5cm³; 0.01M) was put in a conical flask which contains oxalic acid (10ml; 4%) and mixture was titrated against 2,6-dichlorophenolindophenol dye to a persistent pink colour endpoint. The quantity of ascorbic acid was calculated using the formula:

$$\text{Ascorbic acid (mg/100g)} = [0.5/V_1] \times [V_2/15\text{ml}] \times [100\text{ml/weight of the sample}] \times 10$$

The quantity of dye consumed (V₁ml) which is equivalent to the amount of ascorbic acid was obtained.

Phenolic content determination

The total amount phenolic compounds of each extract was determined in duplicates using Folin- Ciocalteu's procedure as described by Sun *et al.*(2007) and Cheng *et al.* (2006).

Ferric reducing assay procedure (FRAP)

Serially diluted solutions were made from the samples (0.2-1mg/ml). About 1ml of the extract was mixed with phosphate buffer (2.5ml; 200mM, pH 6.6) and potassium ferricyanide (2.5cm³; 1%. The tubes were heated in boiling bath for 20min at 50°C, cooled rapidly and mixed

Table 1: pH and total acidity, ascorbic acid and total phenolic compounds of samples

Samples	pH	Total Acidity (mmol/L)	Ascorbic acid (mg/100g)	Total phenolics Content (mg GAE/ml)
Red Onions	5.65	0.150	229.098±0.92	14.25±0.35
White Onions	5.69	0.120	207.841±0.91	6.50±0.35
Garlic	6.94	0.105	191.894±0.93	5.00±0.35

Table 2: The absorbance of the reaction mixture at 700nm with increase in concentration

Concentration mg/ml	Absorbances 700nm		
	Red Onions	White Onions	Garlic
0.2	0.272	0.192	0.320
0.4	0.318	0.240	0.521
0.6	0.400	0.301	0.821
0.8	0.448	0.368	1.100
1	0.520	0.420	1.230

Table 3: The Antioxidant activity of red onions, white onions and garlic.

Samples	Concentration (ug/ml)					
	Absorbance	50	100	200	400	600
Red Onions	Initial	0.447	0.447	0.447	0.447	0.447
	Final	0.300	0.258	0.225	0.215	0.185
	% Scavenging Activity	32.89	42.28	49.66	51.90	58.61
White Onions	Final	0.310	0.268	0.235	0.226	0.198
	% Scavenging Activity	30.65	40.05	47.43	49.44	55.71
Garlic	Final	0.321	0.275	0.260	0.247	0.215
	% Scavenging Activity	28.19	38.48	41.83	44.74	51.90
Ascorbic Acid (Standard)	Final	0.298	0.240	0.200	0.180	0.150
	% Scavenging Activity	33.33	46.31	55.26	59.73	66.44

with trichloroacetic (2.5cm³; 10%) and ferric chloride (0.5cm³; 0.1%). The amount of iron (II) ferricyanide complex formed was estimated spectrophotometrically at 700nm after 10 min.

Antioxidant potential determination

The ability of each of the samples (red onions, white onions and garlic) to inhibit auto oxidation was estimated by measuring its scavenging effect on the DPPH. Dilution of the extract sample (50µg/ml to 800µg/mL) were prepared in methanol. In clean and labeled test tubes, 2mL of DPPH solution (0.002% in methanol) was mixed with 2mL of different concentrations of samples separately. The tubes were incubated at room temperature in dark for 30 minutes and the optical density was measured at 517 nm using UV-Vis Spectrophotometer. The absorbance of the DPPH control was also noted. The scavenging activity of the sample was calculated using the formula:

$$\text{Scavenging activity (\%)} = [(A - B) / A] \times 100$$

Where A is the absorbance of DPPH and B is the absorbance of DPPH and sample combination.

RESULTS

The results of the analysis of pH, Total acidity, Ascorbic acid content and total phenolic compounds in the studied samples are shown in tables 1-3 below.

DISCUSSION

The pH of different samples (10%) ranged from 5.65 to 6.94 and was found to be least in red onions when compared to garlic and white onions (pH of red onions < white onions < garlic). Present research showed that red onion extract exhibited the highest total acidity when compared to other samples. Hence red onions are more effective in retarding oxidation than white onions and garlic.

The result of ascorbic acid content determination in the samples showed 191.894±0.91, 207.841±0.91 and 229.098±0.92mg/100g for white onions, garlic and red onions respectively. The highest amount of ascorbic acid was found in red onions extract but there was no good

linear correlation between ascorbic acid and free radical scavenging activity (table 2). Ascorbic Acid has four-OH groups that can donate hydrogen to an oxidizing system. Because the -OH groups (2 pairs of 2) are on adjacent carbon atoms, ascorbic acid is able to chelate metal ions (Fe^{++}). It also scavenges free radicals, quenches singlet oxygen and acts as a reducing agent. According to Bahorun *et al* (2004), it is normal when ascorbic acid do not correlate with the free radical scavenging activities since ascorbic acid made little or no contribution to the total antioxidant activities of vegetables.

Result of evaluation of total phenolic content showed that examined *allium* extracts differed significantly. Highest phenolic content was evaluated in red onions (14.25 ± 0.35 mg GAE/ml) and lower amount was evaluated in white onions (6.50 ± 0.35 mg GAE/ml). Garlic extract was found to contain the lowest phenolic content among the examined extracts (5.00 ± 0.35 mg GAE/ml.). The reactivity of antioxidants against free radicals is characterized by bond

Dissociation energy (BDE) of the O-H bonds in the phenolic groups of these antioxidants (Atoui *et al*, 2005). Earlier literature (Drozd *et al*. 2011) indicated that raw garlic bulb possessed richer qualitative and quantitative composition of phenolic acids than raw bulbs of onion. Different phenolic compounds earlier found in garlic were anthocyanins and flavonoids while onions majorly contained quercetin, anthocyanins (Benkebila 2007, Nuutila *et al*. 2003)

The linear relationship exist between total phenolic content and radical scavenging activity by DPPH assay (table 2) which supports previous study by Lanzotti (2006) that phenols are mainly responsible for destruction of free radicals by acting as reducing agents, hydrogen donors and singlet oxygen quenchers. An earlier study indicated that the total phenolic content of red onions is higher than white onions and garlic (Deutch, 2003).

In Ferric reducing assay, the absorbance of the reaction mixture at 700nm was found to increase with the concentration of red onions, white onions and garlic, which indicates reducing potential of the samples (table 2). The highest reducing potential was shown by garlic in this present study.

There was no correlation between ferric reducing capacity and radical scavenging activity (tables 2 and 3). The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power of garlic was found to increase with rising concentrations and was found to be two times higher than that of red and white variety.

When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, it gives rise to

the reduced form of DPPH. This assay measures by spectrophotometer the ability of an antioxidant to reduce 2,2-diphenyl-1-picrylhydrazyl. The DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activities of red onions, white onions and garlic are shown in table 3. The result clearly shows that red onions have more capability to destroy or capture free radicals than white onions and garlic. There was also a linear correlation between radical scavenging activity and total phenolic compound. At a concentration of 800ug/ml, the extract of *red onions* scavenged 66.45% of DPPH used for the assay while garlic extract had the lowest (59.73%) DPPH antioxidant activity and when compared with ascorbic acid, a well known antioxidant, the highest scavenging capacity (77.63%) was observed at the concentration of 800ug/ml. A study of the crude ethanolic extract of Nigerian cultivar of garlic with regards to its antioxidant and antimicrobial properties by Ifesan and co-worker (2014) showed that the potency of the spice as antioxidant was satisfactory.

Free radicals are implicated in many health disorders such as neurodegenerative diseases, cancer and AIDS and antioxidants are useful for the management of those diseases. Ascorbic acid is highly bioavailable and is consequently the most important water-soluble antioxidant vitamin in cells, effectively scavenging reactive oxygen species (ROS). Ascorbic acid acts as a chain breaking antioxidant and disrupts the formation of free radicals in the bid to form intracellular materials in the body (Aqil *et al*, 2006).

Phenolic compounds are the major group contributing to the antioxidant activity of vegetables, fruit, cereals and other plant-based materials. The antioxidant activity of the compounds is partly due to one electron reduction potential that is the ability to act as hydrogen or electron donors (Chan, 2007, Atoui *et al*.2005).

CONCLUSION

The antioxidant ability, phenolic content, total acidity and ascorbic acid content of methanol extracts of red onion, white onion and garlic Nigerian cultivars were studied. The results of the study indicate that red onion (*Allium cepa L.*) possess higher total phenolic content than garlic (*Allium sativum L.*). The antioxidant activity of methanol extract of red onions is greater than that of white onions and garlic. The results suggested that red onions followed by white onions were rich sources of polyphenols with promising antioxidant, free radical scavenging activities and ability to provide protection against cell damage.

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