

## Total Antioxidant Potential of Some Selected Beverages Consumed in Lagos State, Nigeria

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### Abstract

Antioxidants have been shown to be beneficial in the maintenance of human health and treatment of some diseases. This study investigated the total antioxidant potential of some selected beverages (green tea, black tea, instant coffee, apple, grape, orange and mango juices, alcoholic herbal bitters, gin, brandy and red wine). Samples were subjected to the following assays 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging activity, nitric oxide scavenging activity, reducing power, total antioxidant capacity and total phenolic content. Results obtained showed a linear relationship for analysis carried out at different concentrations (DPPH, Nitric Oxide and Reducing Power) and confirmed the antioxidant properties of the samples. Brandy displayed the best DPPH scavenging activity of 85.70% at 100 µg/ml and green tea had the best nitric oxide scavenging activity of 83.36% at 100 µg/ml. Grape juice was observed to have superior reducing power of 0.264 mgAAE/100g, 0.423 mg AAE/100g and 0.534 mgAAE/100g at the respective concentrations of 50 µg/ml, 75 µg/ml and 100 µg/ml. Black tea had the highest phenolic content of 61.24 mg/g while grape juice had the highest antioxidant capacity 70.18 mg/100g. The result obtained from study confirmed fruit juices, teas, wine and coffees as good dietary sources of antioxidants.

**Keywords:** Antioxidant Potential; DPPH; Beverages; Reducing Power

### Introduction

In recent times, the trend of life conditions have led to emphasis being laid on intake of biologically-active food substances/dietary components being more important than energy intake. This stems from the fact that a number of diseases specific to the current civilization and an ever-decreasing immunity have led to the development of new therapeutic methods which employ food-drug use in the treatment or prevention of different diseases [1]. Epidemiological studies analyzing the health implications of dietary components rely on intake estimates in sample populations found in databases that list the components in commonly consumed foods. However, it is not clear which dietary components are responsible for the treatment or prevention of these diseases but antioxidants have been shown to be a major player as a result of their protective effect against diseases [2]. Exposure to free radicals from a variety of sources has led organisms (both plants and animals), to develop a series of defense mechanisms to combat the excessive levels of reactive species which cause oxidative stress either by utilizing antioxidants naturally generated in situ in the body (endogenous antioxidants) or externally supplied through foods and supplements as dietary components (exogenous antioxidants) [3-5]. However, the production and effectiveness of endogenous antioxidants in human cells is very much limited and declines overtime with age. Also, when antioxidant defenses are weakened, body cells and tissues become prone to develop dysfunction/disease. Hence, it becomes essential to maintain adequate antioxidant levels without overdosing by complementing with dietary sourced antioxidants [1,6,7]. With a growing interest in research on natural antioxidants, plants come under focus because they have been found to have high concentrations of antioxidant compounds such as polyphenols, carotenoids, flavonoids, some vitamins and enzymes with antioxidant activity. Some of these compounds remain present although at varying concentrations in beverages produced from plants and hence, give the beverages some beneficial attributes when consumed [1]. The objective of this study is to examine if the total antioxidant potential assay is suitable for characterizing the antioxidant properties of commonly marketed Lagos state fruit juices, hot drinks and beverages to obtain robust data useful for determining the potential intake of antioxidants in Nigeria population studies as well as contributing to a better understanding of the role of different antioxidant juice ingredients, also assess the free radical scavenging abilities of the aqueous extracts using four different in vitro assay. This present study was carried out to evaluate total antioxidant potentials of selected beverages consumed in Lagos State which may be helpful to categorize foods according to antioxidant activity and to supplement the already existing food database.

## Materials and Methods

### Sample collection and preparation

Beverages selected for this study include fruit juices, coffee, tea and alcoholic drinks. Four different varieties were selected for the fruit juice and alcoholic drink with the fruit juice including mango, grape, orange and apple juices while the alcoholic drinks included brandy, gin, red wine and alcoholic herbal bitters. Two different product brands were selected for instant coffee, and for the tea, black and green tea was selected. All items were purchased from different points of the Boundary market in Ajegunle, Lagos state, Nigeria. Tea and coffee samples were prepared by infusing 20g of each sample in 100 ml of 90% v/v methanol and left to stand for 12 hrs at room temperature to allow extraction. The infusions were filtered with filter paper, and the filtrates collected in beakers were concentrated over a water bath. Extracts obtained were used in preparing aqueous solutions of 0.1g to 100 ml of distilled water, and from these aqueous solutions, dilutions of 1 ml sample in 5 ml of distilled water were prepared for use. From the fruit juices and alcoholic drinks a direct dilution of 1 ml sample in 5 ml of distilled water was also prepared for use in the analysis.

### Chemicals

All organic solvents and chemicals used in this study were of analytical grade from Sigma, Poole, UK and BDH Laboratory Supplies, UK.

### DPPH radical scavenging activity assay

DPPH radical scavenging activity of the samples was estimated as described by Burits and Bucar [8]. An aliquot of 0.5 ml of the extract in ethanol (95%) at different concentrations (25, 50, 75, 100 µg/ml) was mixed with 2.0 ml of reagent solution (0.004g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes, the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the expression:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where  $A_0$  is the absorption of the blank sample;  $A_1$  is the absorption of the extract.

### Nitric oxide scavenging activity assay

Four mill (4 mls) of each sample at different concentrations (25, 50, 75, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into the test tubes and they were incubated for 2 hrs at 30°C. 2 ml was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthyl ethylenediamine dihydrochloride in 2%  $H_3PO_4$ ). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with naphthyl ethylenediamine was measured at 550 nm [9]. Ascorbic acid was used as standard. The percentage (%) inhibition activity was calculated from the following equation:

$$\% \text{ inhibition} = [(A_0 - A_1) / A_0] \times 100$$

Where,  $A_0$  is the absorbance of the Control;  $A_1$  is the absorbance of the extract.

### Reducing power assay

The reducing property of the samples was determined as described by Pulido., et al [10]. Various concentrations of the samples (25 to 100 µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50°C for 20 minutes. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. the upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (25 to 100 µg/ml) was used as standard.

$$\text{Reducing power} = (A_{\text{test}} / A_{\text{blank}}) - 1 \times 100$$

$A_{\text{test}}$  is absorbance of test solution;  $A_{\text{blank}}$  is absorbance of blank.

### Total antioxidant capacity determination

The total antioxidant capacity of the extracts was determined using the method of Prieto., et al [11]. A sample of the extract (0.3 ml) was mixed with 3 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and incubated in a boiling water bath at 95°C for 90 minutes. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm. The total antioxidant capacity was expressed as equivalent of ascorbic acid.

### Estimation of total phenolic content

The amount of total phenolic content was determined by Folin-Ciocalteu reagent method using gallic acid as a standard. 0.5 ml samples and 0.1 ml of Folin-Ciocalteu reagent (0.5 N) were mixed and incubated at room temperature for 15 minutes. 2.5 ml sodium carbonate solution (7.5% w/v) was added with further incubation for another 30 minutes at room temperature. The absorbance of the solution was measured at 760 nm. The concentration of total phenol was expressed as gallic acid equivalent (GAE) (mg/g of dry mass) which is a commonly used reference value [12].

### Statistical analysis

The conventional statistical methods were used to calculate means and standard deviations. All the measurements were performed in duplicate and the data are presented as mean  $\pm$  standard deviation (SD). Data were statistically evaluated by use of one-way ANOVA using 7.5 version of the SPSS computer software. The values were considered significant at  $P < 0.05$ . Graphpad prism 5.0 software (Graph pad prism Software Inc., San Diego, CA, USA) was used for the descriptive statistics.

## Results and Discussion

### Scavenging activity towards DPPH free radicals

Several research works has been carried out on fruit juice, beverages and hot drinks and they have received great because they contain high amounts of known antioxidants such as polyphenols, vitamin C, vitamin E, Maillard reaction products,  $\beta$ -carotene, and lycopene. The consumption of fruit juices, beverages and hot drinks has been reported by several researchers to be inversely associated with morbidity and mortality from degenerative diseases [2,13]. Antioxidants are usually added to foods to prevent the formation of free radicals which may slow down the oxidation process by inhibiting the initiation process and propagation steps which may lead to the termination of reaction (Shahidi & Amarowicz, 1994). These antioxidants are mostly used to retard formation of these reactive oxygen species so as to maintain nutritional quality and shelf-life of foods (Jadhav, *et al.* 1996). The results of radical scavenging activity of samples towards stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical as analyzed against the reference antioxidant ascorbic acid are shown in figures 1-3. It clearly shows that as concentration increases from 25  $\mu\text{g/ml}$  to 100  $\mu\text{g/ml}$ , samples exhibit increasing activity. Sample AB3 showed the highest scavenging activity at a concentration of 100  $\mu\text{g/ml}$ .

Sample	25 $\mu\text{g/ml}$	50 $\mu\text{g/ml}$	75 $\mu\text{g/ml}$	100 $\mu\text{g/ml}$
T <sub>1</sub>	47.34 $\pm$ 0.16	53.81 $\pm$ 0.39	56.62 $\pm$ 0.46	70.32 $\pm$ 0.30
T <sub>2</sub>	50.00 $\pm$ 0.38	55.94 $\pm$ 0.23	61.26 $\pm$ 0.38	74.36 $\pm$ 0.08
C <sub>1</sub>	52.44 $\pm$ 0.23	59.67 $\pm$ 0.31	67.89 $\pm$ 0.16	75.57 $\pm$ 0.38
C <sub>2</sub>	47.64 $\pm$ 0.46	51.75 $\pm$ 0.15	59.36 $\pm$ 0.61	69.18 $\pm$ 0.23
J <sub>1</sub>	49.24 $\pm$ 0.23	52.82 $\pm$ 0.31	63.02 $\pm$ 0.46	69.26 $\pm$ 0.16
J <sub>2</sub>	50.53 $\pm$ 0.15	55.02 $\pm$ 0.23	62.03 $\pm$ 0.23	72.98 $\pm$ 0.23
J <sub>3</sub>	48.94 $\pm$ 0.39	55.48 $\pm$ 0.38	64.84 $\pm$ 0.30	74.59 $\pm$ 0.31
J <sub>4</sub>	55.41 $\pm$ 0.16	60.50 $\pm$ 0.23	68.88 $\pm$ 0.08	79.45 $\pm$ 0.15
AB <sub>1</sub>	53.65 $\pm$ 0.23	63.55 $\pm$ 0.23	70.63 $\pm$ 0.31	80.22 $\pm$ 0.31
AB <sub>2</sub>	55.10 $\pm$ 0.31	62.10 $\pm$ 0.30	75.65 $\pm$ 0.16	83.26 $\pm$ 0.15
AB <sub>3</sub>	56.39 $\pm$ 0.23	65.37 $\pm$ 0.23	72.30 $\pm$ 0.15	85.70 $\pm$ 0.31
AB <sub>4</sub>	54.95 $\pm$ 0.16	67.13 $\pm$ 0.31	76.33 $\pm$ 0.38	84.86 $\pm$ 0.23
Ascorbic	58.30 $\pm$ 1.07	67.73 $\pm$ 0.61	79.23 $\pm$ 4.95	86.68 $\pm$ 0.53

**Table 1:** Percentage (%) inhibition towards DPPH free radicals

Values represented as mean  $\pm$  standard deviation ( $N = 2$ ). J<sub>1</sub>: Apple Juice, AB<sub>1</sub>: Herbal Bitters; T<sub>1</sub>: Green Tea; J<sub>2</sub>: Grape Juice; AB<sub>2</sub>: Dry Gin; T<sub>2</sub>: Black Tea; J<sub>3</sub>: Orange Juice; AB<sub>3</sub>: Brandy; J<sub>4</sub>: Mango Juice; AB<sub>4</sub>: Red Wine; C: Instant Coffee.

The order of effectiveness of fruit juice inhibiting free radicals is given as orange juice > apple juice > grape juice > mango juice while the order of effectiveness of tea inhibiting free radical is given as green > instant coffee > black tea > coffee with wine given as herbal biters > red wine > dry gin > brandy.

It is a known fact that apple juice is a rich source of flavonoids and other phenolic in the human diet [14]. It has also been reported by several researchers that high antioxidant capacity of fruit juices is likely to be as a result of their high contents of phenolics and flavonoids such as anthocyanins [15,16], which have been demonstrated to produce strong antioxidant activities in different model systems [17,18]. Positive health benefits of the consumption of fruit juice include improvement in the endothelial function, increase in the serum anti-

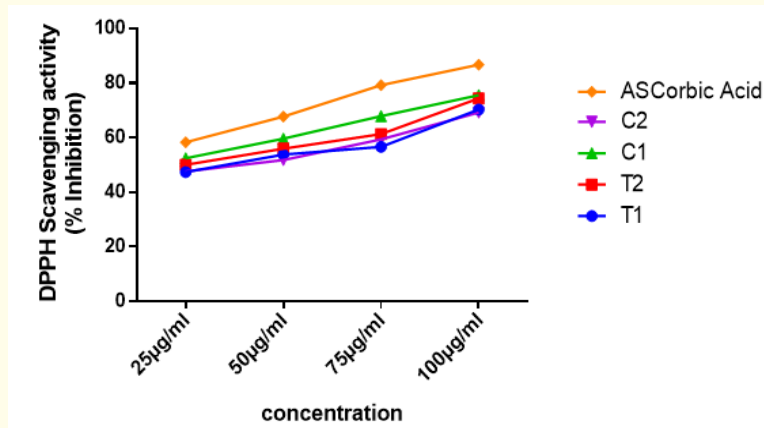


Figure 1: DPPH radical scavenging activity of coffee and tea.

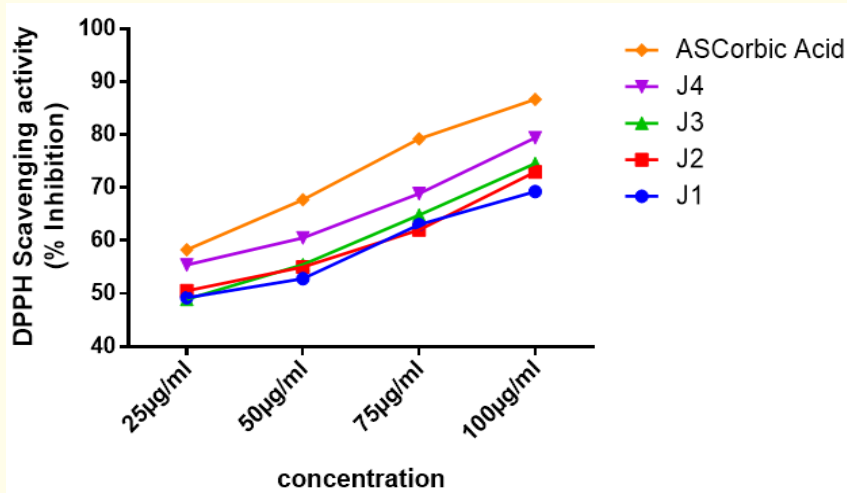


Figure 2: DPPH radical scavenging activity of juice samples.

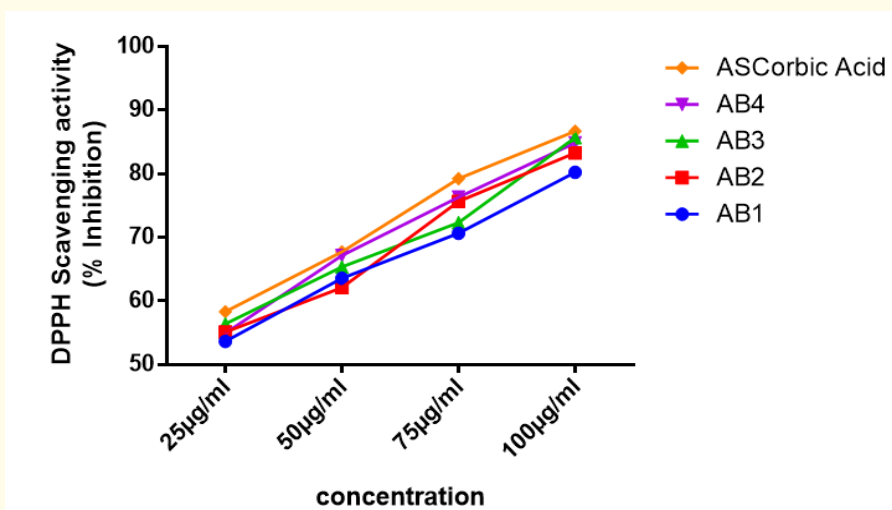


Figure 3: DPPH radical scavenging activity of alcoholic beverage samples.

oxidant capacity, protection of LDL against oxidation, decrease in native plasma protein oxidation, and reduction in platelet aggregation [16]. This result is in agreement with the higher concentration of phenolic compounds, carotenoids and vitamin C present in these juices [13,19,20]. The differences observed in the antioxidant activities among fruit juices could be attributed to their differences in phenolic contents and compositions and to other non-phenolic antioxidants present in the samples. DPPH radical is widely used as the model system to investigate the scavenging activities of several natural compounds [21]. DPPH is scavenged by antioxidants through the donation of proton forming the reduced DPPH which can be quantified by its decrease of absorbance [22]. Radical scavenging activity increased with increasing percentage of the free radical inhibition [21]. From the scavenging activity of samples towards the DPPH radicals, it was observed that there was a linear relationship between the increasing sample concentrations and percentage inhibition of radicals (i.e. as concentration increases so also does the inhibition of radicals). This agrees with the findings of Mohamed [2] who carried out a similar research in Egypt using almost similar samples. The difference observed in the percentage inhibition between the coffee samples could be as a result of the different manufacturing process of both samples.

### Scavenging activity towards nitric oxide radical

Radical scavenging activity is very crucial to the survival of living organisms, due to the deleterious role of free radicals in foods and biological systems. Routinely, antioxidant potencies of plants are assessed by their ability to scavenge DPPH, an unstable compound which turns to a stable diamagnetic molecule when protonated. This stability is visually noticeable as a discoloration from purple to golden yellow. Figures 4-6 show the nitric oxide scavenging activity assay at different concentrations. It was observed that T1 exhibited highest activity (83.36%) at the highest concentration of 100 µg/ml and also the least activity (41.61%) at a concentration of 25 µg/ml (Table 2). It was also observed that at sample concentration of 50 µg/ml, AB3 (64.48%) and AB4 (66.78%) showed higher activity than ascorbic acid (63.40%) which was the reference sample. Nitric oxide scavenging activity increased with increasing sample concentration.

Sample	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
T <sub>1</sub>	41.61 ± 0.07	50.41 ± 0.21	64.48 ± 0.20	83.36 ± 0.14
T <sub>2</sub>	46.41 ± 0.27	55.55 ± 0.20	64.14 ± 0.27	79.50 ± 0.07
C <sub>1</sub>	48.85 ± 0.14	55.75 ± 0.27	64.82 ± 0.14	82.95 ± 0.14
C <sub>2</sub>	45.54 ± 0.07	46.28 ± 0.14	66.78 ± 0.20	70.64 ± 0.27
J <sub>1</sub>	48.17 ± 0.27	56.03 ± 0.14	65.63 ± 0.27	71.05 ± 0.14
J <sub>2</sub>	49.94 ± 0.14	54.47 ± 0.34	64.62 ± 0.21	70.57 ± 0.07
J <sub>3</sub>	47.64 ± 0.14	61.85 ± 0.14	66.92 ± 0.21	73.82 ± 0.21
J <sub>4</sub>	52.71 ± 0.21	57.38 ± 0.14	69.35 ± 0.07	75.17 ± 0.20
AB <sub>1</sub>	51.15 ± 0.27	60.42 ± 0.20	68.88 ± 0.27	75.65 ± 0.14
AB <sub>2</sub>	48.85 ± 0.14	62.93 ± 0.14	70.91 ± 0.27	81.06 ± 0.14
AB <sub>3</sub>	47.64 ± 0.14	64.48 ± 0.20	69.69 ± 0.27	83.09 ± 0.14
AB <sub>4</sub>	52.17 ± 0.21	66.78 ± 0.07	70.23 ± 0.27	82.21 ± 0.07
Ascorbic	54.94 ± 1.49	63.40 ± 2.24	79.23 ± 1.29	85.52 ± 2.84

**Table 2:** Percentage (%) inhibition towards nitric oxide radicals.

Values represented as mean ± standard deviation (N = 2). J<sub>1</sub>: Apple Juice; AB<sub>1</sub>: Herbal Bitters; T<sub>1</sub>: Green Tea; J<sub>2</sub>: Grape Juice; AB<sub>2</sub>: Dry Gin; T<sub>2</sub>: Black Tea; J<sub>3</sub>: Orange Juice; AB<sub>3</sub>: Brandy; J<sub>4</sub>: Mango Juice; AB<sub>4</sub>: Red Wine; C: Instant Coffee.

Phenolics and flavonoids are commonly known to exhibit anti-allergic, anti-inflammatory, anti-microbial and anticancer activity (Balch and Balchi, 2000; Jisika, *et al.* 1992). From the foregoing, it would be rational to expect that the extract with higher phyto-chemical content would exhibit a stronger antioxidant effect.

Nitric oxide is a potential pleiotropic mediator of various physiological processes such as smooth muscle relaxation, transmission neuronal signal, inhibition of platelet aggregation and regulation of cell-mediated toxicity [23]. The nitric oxide scavenging activity of the samples is observed to be concentration dependent (i.e. as concentration increases activity equally increases) thus, following the trend set by DPPH scavenging activity. At the concentration of 50 µg/ml, AB<sub>4</sub> had an inhibition 66.78% which is higher than ascorbic acid inhibition of 63.4%, clearly indicating that AB<sub>4</sub> has maximum activity at this concentration but will still function at other concentrations. The results demonstrates the ability of the samples in arresting the chain of reactions initiated by excess generation of reactive nitrogen species (RNS) that are deleterious to the human health [24] and offers credence to the possibility of using samples as pharmacological remedies to ameliorate complications of diabetes and cardiovascular diseases as a result of their antioxidant properties.

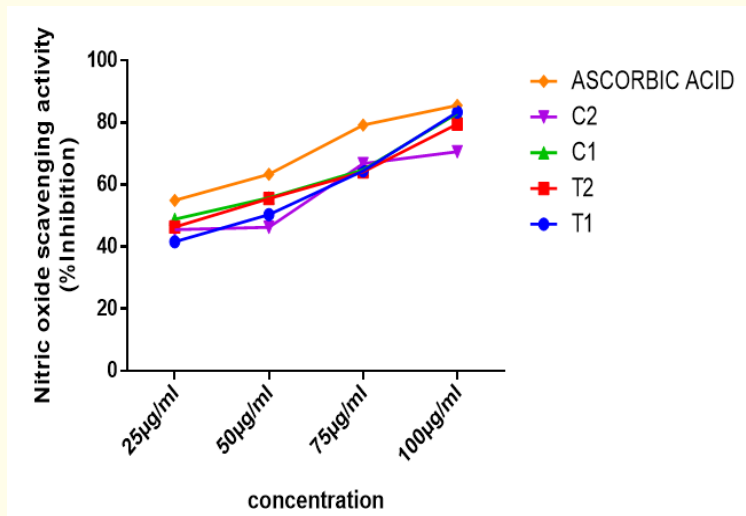


Figure 4: Nitric Oxide scavenging activity of coffee and tea.

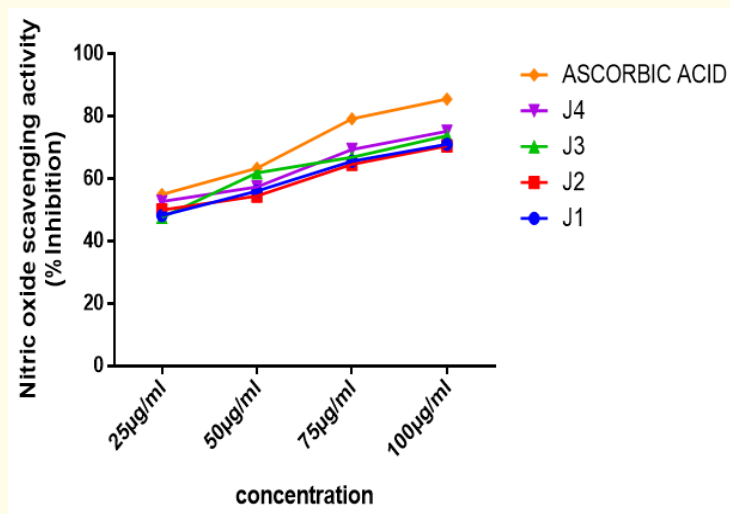


Figure 5: Nitric oxide scavenging activity of juice sample.

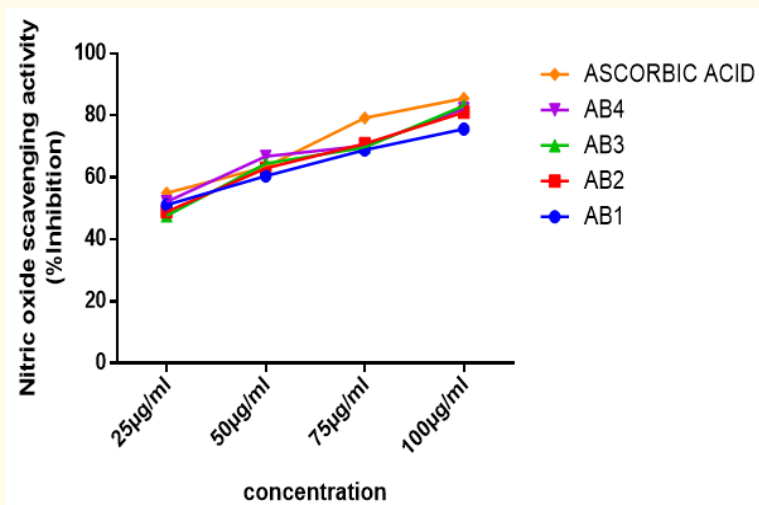


Figure 6: Nitric oxide scavenging activity of alcoholic beverages samples.

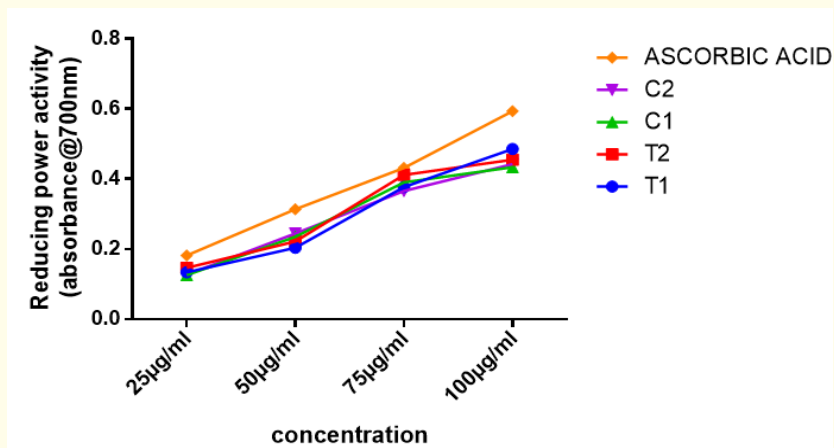
**Reducing power property**

From the results obtained in figures 8 and 9 at the least concentration of 25 µg/ml, T1 showed peak activity of inhibition whereas sample J2 had peak activity over all other samples at concentrations 50 µg/ml, 75 µg/ml and 100 µg/ml as determined against a reference antioxidant.

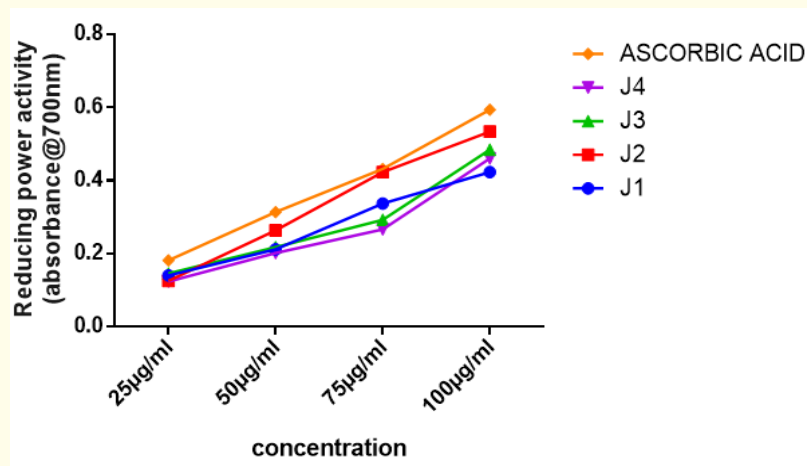
Sample	25 µg/ml	50 µg/ml	75 µg/ml	100 µg/ml
T <sub>1</sub>	0.134 ± 0.001	0.204 ± 0.001	0.377 ± 0.001	0.486 ± 0.002
T <sub>2</sub>	0.147 ± 0.002	0.222 ± 0.002	0.412 ± 0.001	0.455 ± 0.003
C <sub>1</sub>	0.126 ± 0.002	0.235 ± 0.001	0.391 ± 0.002	0.434 ± 0.003
C <sub>2</sub>	0.127 ± 0.002	0.245 ± 0.002	0.366 ± 0.002	0.443 ± 0.001
J <sub>1</sub>	0.141 ± 0.002	0.212 ± 0.002	0.337 ± 0.001	0.423 ± 0.001
J <sub>2</sub>	0.126 ± 0.001	0.264 ± 0.002	0.423 ± 0.002	0.534 ± 0.001
J <sub>3</sub>	0.146 ± 0.001	0.218 ± 0.002	0.293 ± 0.002	0.484 ± 0.001
J <sub>4</sub>	0.124 ± 0.002	0.202 ± 0.001	0.267 ± 0.002	0.461 ± 0.002
AB <sub>1</sub>	0.140 ± 0.002	0.214 ± 0.001	0.248 ± 0.001	0.375 ± 0.001
AB <sub>2</sub>	0.118 ± 0.001	0.167 ± 0.002	0.237 ± 0.002	0.384 ± 0.002
AB <sub>3</sub>	0.105 ± 0.001	0.145 ± 0.003	0.265 ± 0.003	0.387 ± 0.003
AB <sub>4</sub>	0.092 ± 0.001	0.182 ± 0.001	0.254 ± 0.002	0.390 ± 0.001
Ascorbic	0.182 ± 0.030	0.314 ± 0.062	0.432 ± 0.031	0.594 ± 0.038

**Table 3:** Percentage (%) reducing power of selected beverages.

Values are expressed as mean ± SJ; J<sub>1</sub>: Apple Juice; AB<sub>1</sub>: Herbal Bitters; T<sub>1</sub>: Green Tea; J<sub>2</sub>: Grape Juice; AB<sub>2</sub>: Dry Gin; T<sub>2</sub>: Black Tea; J<sub>3</sub>: Orange Juice; AB<sub>3</sub>: Brandy; J<sub>4</sub>: Mango Juice; AB<sub>4</sub>: Red Wine; C: Instant Coffee.



**Figure 7:** Reducing power activity of coffee and tea.



**Figure 8:** Reducing power activity of juice samples.



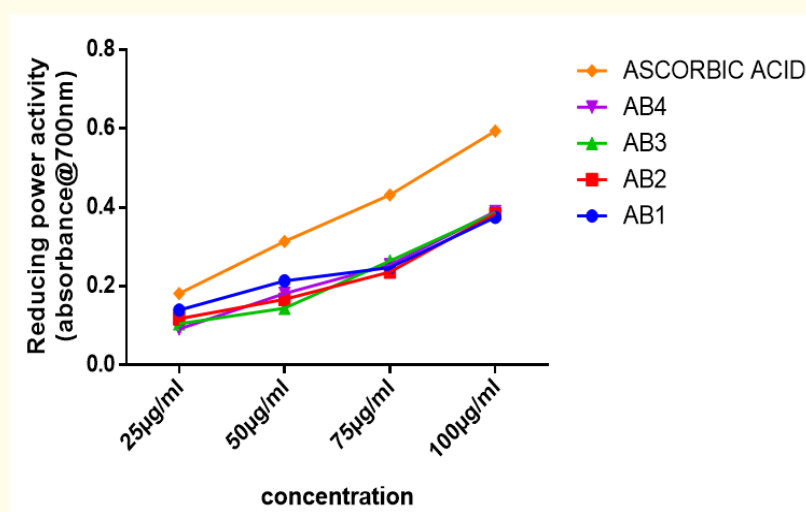


Figure 9: Reducing power activity of juice samples.

Reducing power is a novel anti-oxidation defense mechanism; the two mechanisms that are available to affect this property are electron transfer and hydrogen atom transfer [25]. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity [26], thus reducing power of samples indicate a high potential in hydrogen-donating ability which could react with free radicals to convert them to more stable products thereby terminating radical chain reactions [27]. Samples showed varying reducing capacity with respect to concentration following the trend that was set in the DPPH scavenging assay. Also, J2 had the best antioxidant property with regards to reducing power because of its consistency of highest values (0.264 mgAAE/100g, 0.423 mgAAE/100g and 0.534 mgAAE/100g) at the respective concentrations of 50 µg/ml, 75 µg/ml and 100 µg/ml and it was also the closest to the values of ascorbic acid which was used as a reference antioxidant. This trend exhibited by the samples implies that they are capable of donating hydrogen atom in a dose dependent manner. The varying values of the samples can be attributed to the presence of compounds with hydroxyl groups, which can readily and effectively function as hydrogen donor. It can also be attributed to the phenolic content profile of the samples taking into consideration the type, quantity and activity of polyphenols in each beverage sample as inferred from literature [28].

### Total antioxidant capacity

Plants are the best sources of active secondary metabolites which are beneficial to mankind. Many plants origin drugs have been reported with biological properties like antibacterial, antifungal, antioxidants, anti-inflammatory and hypoglycemic [29]. Antioxidant activity is a basic function important for life from which biological functions such as antimutagenicity, anti-carcinogenicity and antiaging originated, thereby preventing the incidence of chronic diseases in man [29]. Table 4 shows the results of total antioxidant capacity assay for each sample. From the table, J<sub>2</sub> has the highest value for total antioxidant capacity compared to AB<sub>2</sub> which has the least total antioxidant capacity value.

The total antioxidant capacity assay is a spectrophotometric method based on the reduction of Mo(VI) to Mo(V) by the sample analyte and the subsequent formation of a green phosphate/Mo(V) complex at acidic pH. Also known as the phosphomolybdenum method, it has been optimized with respect to linearity interval, repetitivity and reproducibility, and molar absorption coefficients for the quantitation of several antioxidants, including vitamin E and is routinely applied in laboratories to evaluate the total antioxidant capacity of plant extracts and to determine vitamin E in a variety of food samples [11]. The results obtained confirm the antioxidant property of the beverages that were investigated with respect to vitamin E. The specificity of the method at 25 - 37°C (temperatures at which other weaker antioxidants are not detected) makes the phosphomolybdenum method a good alternative for the determination of vitamin E in a variety of samples (plant lipid-soluble extracts, vegetal oils, butter, pharmaceutical and cosmetic preparations, human serum, etc). Other compounds that might contribute to the total lipid antioxidant capacity include carotenoid, flavonoid, and cinnamic acid derivatives [11].

### Total phenolic content

Plant phenols which consist of simple phenolic acids, flavonoids, stilbenes and other polyphenolic compounds, have hydroxyl groups conjugated to an aromatic hydrocarbon group. The bio-mechanisms of their reaction which include anti-oxidation, anti-inflammation, carcinogen detoxification and cholesterol reduction of plant phenols have been shown to cause reduction in risk of several chronic diseases [29]. It has also been reported that there is a relationship between health protective benefits of phenolic compounds and their antioxidant properties [29]. Table 5 shows an estimation of the total phenolic content of the samples. It was observed that C<sub>2</sub> and J<sub>1</sub> have



S/N	Sample	Total Antioxidant
1	T <sub>1</sub>	37.91 ± 0.35
2	T <sub>2</sub>	39.67 ± 0.18
3	C <sub>1</sub>	42.24 ± 0.58
4	C <sub>2</sub>	55.76 ± 0.28
5	J <sub>1</sub>	70.18 ± 0.23
6	J <sub>2</sub>	78.68 ± 0.23
7	J <sub>3</sub>	29.83 ± 0.35
8	J <sub>4</sub>	52.86 ± 0.35
9	AB <sub>1</sub>	28.11 ± 0.23
10	AB <sub>2</sub>	17.41 ± 0.35
11	AB <sub>3</sub>	49.02 ± 0.23
12	AB <sub>4</sub>	21.78 ± 0.18

**Table 4:** Total antioxidant capacity (mg/100g) of some beverage samples.  
Values represented as mean ± standard deviation (N = 2).

S/N	Sample	Total phenolic content mg/g
1	T <sub>1</sub>	49.93 ± 0.28
2	T <sub>2</sub>	61.24 ± 0.38
3	C <sub>1</sub>	41.22 ± 0.33
4	C <sub>2</sub>	50.59 ± 0.22
5	J <sub>1</sub>	19.09 ± 0.39
6	J <sub>2</sub>	44.62 ± 0.16
7	J <sub>3</sub>	25.41 ± 0.28
8	J <sub>4</sub>	26.74 ± 0.16
9	AB <sub>1</sub>	29.71 ± 0.28
10	AB <sub>2</sub>	25.02 ± 0.38
11	AB <sub>3</sub>	34.12 ± 0.33
12	AB <sub>4</sub>	28.97 ± 0.33

**Table 5:** Total phenolic content (mg/g) of selected beverage samples.

the highest and lowest total phenolic content respectively. It can be observed that there is an occurrence of disparity between the coffee samples which is statistically significantly ( $P < 0.05$ ). This can be accounted for by the producer processing methods for each brand. T2 was also shown to have a higher phenolic content that is statistically significant compared to T1. This is contrary to the findings of Li., *et al.* [30], Lubomila., *et al.* [31], Oboh and Omoregie [32] that green tea had higher phenolic content compared to black tea. The total phenolic content recorded for AB3 could be as a result of phenols seeping into the sample from wooden casks during the aging of brandy. Many factors influence the levels of polyphenols and antioxidants in food. One set of factors arises on the farm and includes plant genetics, farming practices, soil fertility, the weather, pest pressure and pest management systems, and harvest time and ripeness. Another set of factors come into play as food leaves the farm and make its way to consumers i.e. how food is processed and stored can alter levels, sometimes dramatically [33].

Plants contain high concentrations of numerous redox-active antioxidants, such as polyphenols, carotenoids, tocopherols, glutathione, ascorbic acid and enzymes with antioxidant activity, which fight against hazardous oxidative damage of plant cell components [1]. Phenolic phytochemicals inhibit auto-oxidation of unsaturated lipids, thus preventing the formation of oxidized low-density lipoprotein (LDL), which is considered to induce cardiovascular disease [34]. The total phenolic content of the samples as determined only gives the quantity of polyphenol and phenolic compounds present in the samples without identifying the specific types and their individual quantities. The different behavior exhibited by teas is due to the changes that occurred during fermentation process and the presence flavanols in green tea leaves which are mainly catechins and their gallic esters may undergo an oxidative polymerization reaction by polyphenol oxidase,

which gives rise to the leaves black colour. During oxidation, majority of the catechin present in the green tea is converted to oxyproducts, such as thearubingens and theaflavins, with a loss of antioxidant capacity [35]. In coffee production, the roasting process will result in significant changes in the chemical composition and biological activities of the coffee bean which may lead to the production of compounds derived from Maillard reaction, carbohydrates caramelization and pyrolysis of organic compounds [36,37]. When coffee is roasted, most polyphenolic compounds are destroyed while Maillard reaction compounds with antioxidant properties will be generated which will lead to an increased antioxidant activity in the -carotene-linoleic acid model system [36,38]. The antioxidant properties are always correlated with the total amount of antioxidants and with the presence of selected compounds. It could then be concluded that total antioxidant potentials of fruit juice, wines and beverages can be said to be a combined action of different endogenous antioxidants which may due to the differences in content and composition of bioactive component and structures of potential phenolic antioxidants present [39-41].

## Conclusion

The validity of the total antioxidant potential (TAP) as an approach for investigating the role of antioxidants in the protective effect of food and drinks is growing. The data presented here confirm that DPPH scavenging assay, Nitric oxide scavenging assay, Reducing power assay, Total phenolic content and Total antioxidant capacity assay are well-founded methods and appropriate for surveying the antioxidant capacities of beverage samples (coffee, tea, some fruit juices and alcoholic beverages). On the whole, the results are partially in good accordance with the literature data, partially not. Obviously, this is because of basically different survey parameters. Coupled with an appropriate questionnaire, this will allow the evaluation of the overall intake of antioxidant-equivalents in selected groups of Nigerian population in relation to the incidence of oxidative stress-induced diseases.

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