Total Antioxidant Potential of Selected Beverages Consumed In Lagos State

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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 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 100µg/ml and green tea had the best nitric oxide scavenging activity of 83.36% at 100µg/ml. Grape juice was observed to have superior reducing power of 0.264mgAAE/100g, 0.423mgAAE/100g and 0.534mgAAE/100g at the respective concentrations of 50µg/ml, 75µg/ml and 100µg/ml. Black tea had the highest phenolic content of 61.24mg/g while grape juice had the highest antioxidant capacity 70.18mg/100g.

Key Word: Ambient Temperature, Turbine Inlet Temperature, Thermal Efficiency, Net Work, Power Output

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1. 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 (Dumbrava *et al.*, 2011). 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 (Mohamed, 2008).

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)(Valko *et al.*, 2007; Lien *et al.*, 2008; Shalaby and Shanab, 2013).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 (Benbrook, 2005; Carlos and Bucalen, 2008; Dumbrava *et al.,* 2011). 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 (Dumbrava *et al.,* 2011; Olatidoye *et al.*, 2015).

2. 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 100ml of 90% v/v methanol and left to stand for 12 hr 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 100ml of distilled water, and from these aqueous solutions, dilutions of 1ml sample in 5ml of distilled water were prepared for use. From the fruit juices and alcoholic drinks a direct dilution of 1ml sample in 5ml 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 (2000). An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75,100µg/ ml) was mixed with 2.0 ml of reagent solution (0.004 g 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 min 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:

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

Where A_0 is the absorption of the blank sample and A_1 is the absorption of the extract.

Nitric Oxide Scavenging Activity Assay

4 ml 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 h at 30 °C. 2 ml was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthylethylenediaminedihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylenediamine was measured at 550 nm (Alisi *et al.*, 2008). Ascorbic acid was used as standard.

The percentage (%) inhibition activity was calculated from the following equation:
% inhibition = $[(A_0-A_1)/A_0] \times 100$

Where, A_0 is the absorbance of the Control and A_1 is the absorbance of the extract.

Reducing Power Assay

The reducing property of the samples was determined as described by Pulido, Bravo, and Saura-Calixto (2000). Various concentrations of the samples (25 to 100µg/ml) in 1.0 ml of deionizedwater were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). The mixture was incubated at 50^0C for 20 min. 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 100µg/ml) was used as standard.

Reducing power = (A_{test}/ A_{blank})-1 X 100
A_{test} is absorbance of test solution; A_{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.*, (1999). 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 950 C for 90 min. 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 phenol content was determined by Folin-Ciocateu 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 (McDonald *et al.*, 2001).

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 ± 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 (Graphpad prism Software Inc., San Diego, CA, USA) was used for the descriptive statistics.

4. RESULTS AND DISCUSSION

Scavenging activity towards DPPH free radicals

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 4.1.1, 4.1.2 and 4.1.3 (see also appendix 1). It clearly shows that as concentration increases from 25µg/ml to 100µg/ml, samples exhibit increasing activity. Sample AB_3 showed the highest scavenging activity at a concentration of 100µg/ml.

Table1: Percentage (%) inhibition towards DPPH free radicals

Sample	25µg/ml		50µg/ml		75µg/ml		100µg/ml	
T_1	47.34	±0.16	53.81	±0.39	56.62	±0.46	70.32	±0.30
T_2	50.00	±0.38	55.94	±0.23	61.26	±0.38	74.36	±0.08
C_1	52.44	±0.23	59.67	±0.31	67.89	±0.16	75.57	±0.38
C_2	47.64	±0.46	51.75	±0.15	59.36	±0.61	69.18	±0.23
J_1	49.24	±0.23	52.82	±0.31	63.02	±0.46	69.26	±0.16
J_2	50.53	±0.15	55.02	±0.23	62.03	±0.23	72.98	±0.23
J_3	48.94	±0.39	55.48	±0.38	64.84	±0.30	74.59	±0.31
J_4	55.41	±0.16	60.50	±0.23	68.88	±0.08	79.45	±0.15
AB_1	53.65	±0.23	63.55	±0.23	70.63	±0.31	80.22	±0.31
AB_2	55.10	±0.31	62.10	±0.30	75.65	±0.16	83.26	±0.15
AB_3	56.39	±0.23	65.37	±0.23	72.30	±0.15	85.70	±0.31
AB_4	54.95	±0.16	67.13	±0.31	76.33	±0.38	84.86	±0.23
Ascorbic	58.30	±1.07	67.73	±0.61	79.23	±4.95	86.68	±0.53

Values represented as mean ± standard deviation (N=2). J_1 = apple juice, AB_1 = Herbal bitters T_1 = green tea J_2 = grape juice, AB_2 = Dry gin, T_2 = black tea, J_3 = orange juice, AB_3 = Brandy, J_4 = mango juice, AB_4 = Red wine, C = instant coffee

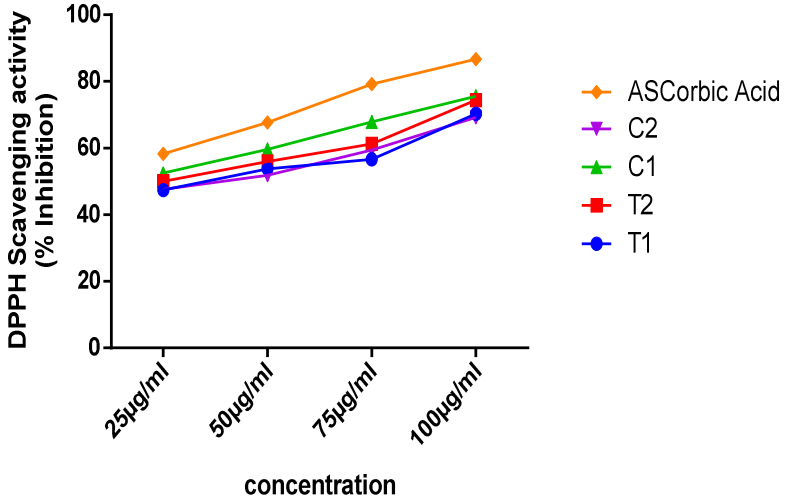

Fig.1: DPPH radical scavenging activity of coffee and tea

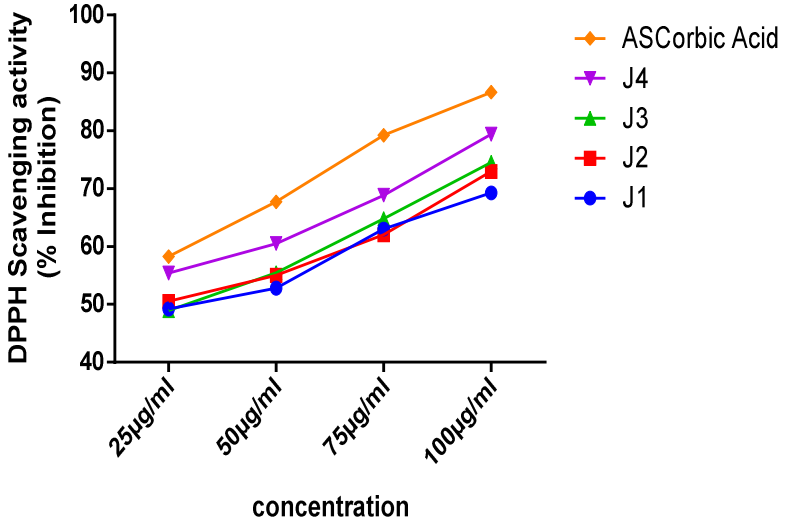

Fig.2 : DPPH radical scavenging activity of juice samples.

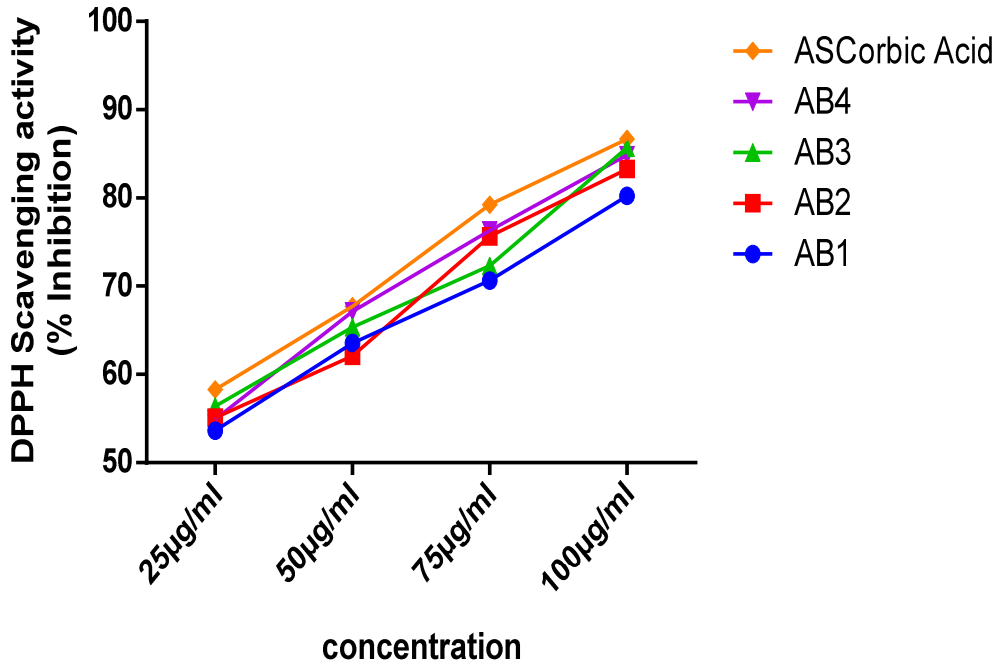

Fig. 3 DPPH radical scavenging activity of alcoholic beverage samples

DPPH radical is widely used as the model system to investigate the scavenging activities of several natural compounds (Bhaskar *et al.,* 2007). DPPH is scavenged by antioxidants through the donation of proton forming the reduced DPPH which can be quantified by its decrease of absorbance (Chowdhury *et al.*, 2011). Radical scavenging activity increased with increasing percentage of the free radical inhibition (Baskar *et al.,* 2007).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 (2008) 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

Figures 1,.2 and .3 show the nitric oxide scavenging activity assay at different concentrations. It was observed that T_1 exhibited highest activity (83.36%) at the highest concentration of 100µg/ml and also the least activity (41.61%) at a concentration of 25µg/ml (Appendix 2). It was also observed that at sample concentration of 50µg/ml, AB_3 (64.48%) and AB_4 (66.78%) showed higher activity than ascorbic acid (63.40%) which was the reference sample. Nitric oxide scavenging activity increased with increasing sample concentration.

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

Sample	25µg/ml		50µg/ml		75µg/ml		100µg/ml	
T_1	41.61	±0.07	50.41	±0.21	64.48	±0.20	83.36	±0.14
T_2	46.41	±0.27	55.55	±0.20	64.14	±0.27	79.50	±0.07
C_1	48.85	±0.14	55.75	±0.27	64.82	±0.14	82.95	±0.14
C_2	45.54	±0.07	46.28	±0.14	66.78	±0.20	70.64	±0.27
J_1	48.17	±0.27	56.03	±0.14	65.63	±0.27	71.05	±0.14
J_2	49.94	±0.14	54.47	±0.34	64.62	±0.21	70.57	±0.07
J_3	47.64	±0.14	61.85	±0.14	66.92	±0.21	73.82	±0.21
J_4	52.71	±0.21	57.38	±0.14	69.35	±0.07	75.17	±0.20
AB_1	51.15	±0.27	60.42	±0.20	68.88	±0.27	75.65	±0.14
AB_2	48.85	±0.14	62.93	±0.14	70.91	±0.27	81.06	±0.14
AB_3	47.64	±0.14	64.48	±0.20	69.69	±0.27	83.09	±0.14
AB_4	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

Values represented as mean ± standard deviation (N=2). J_1 = apple juice, AB_1 = Herbal bitters ,T_1 = green tea, J_2 = grape juice, AB_2 = Dry gin, T_2 = black tea, J_3 = orange juice, AB_3 = Brandy, J_4 = mango juice, AB_4 = Red wine, C = instant coffee

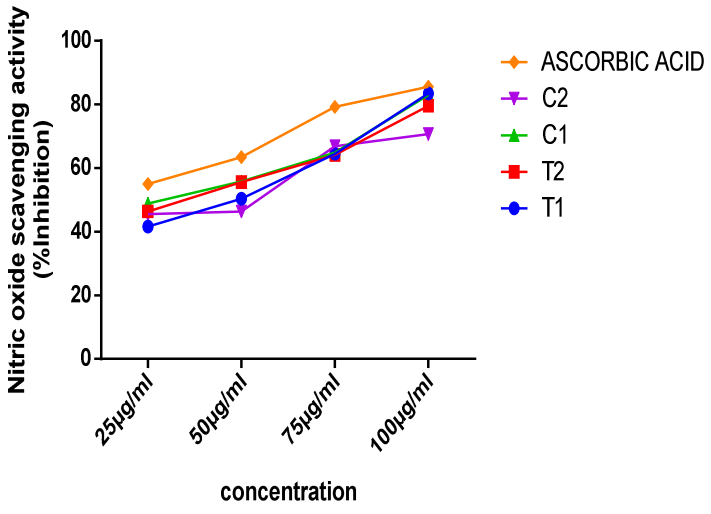

Fig. 4.2.1 Nitric Oxide scavenging activity of coffee and tea

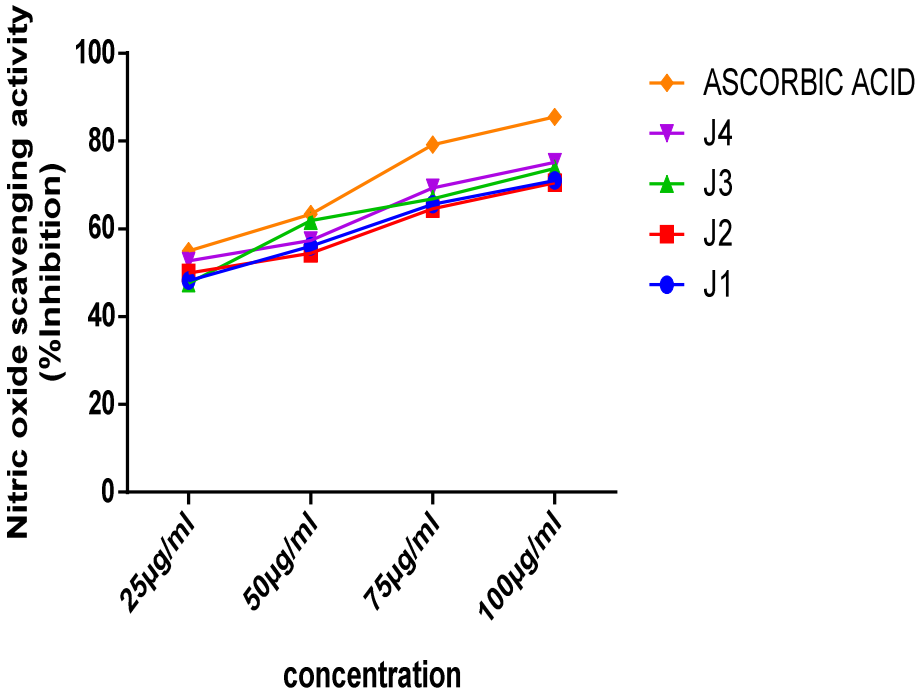

Fig. 4: Nitric Oxide scavenging activity of juice sample.

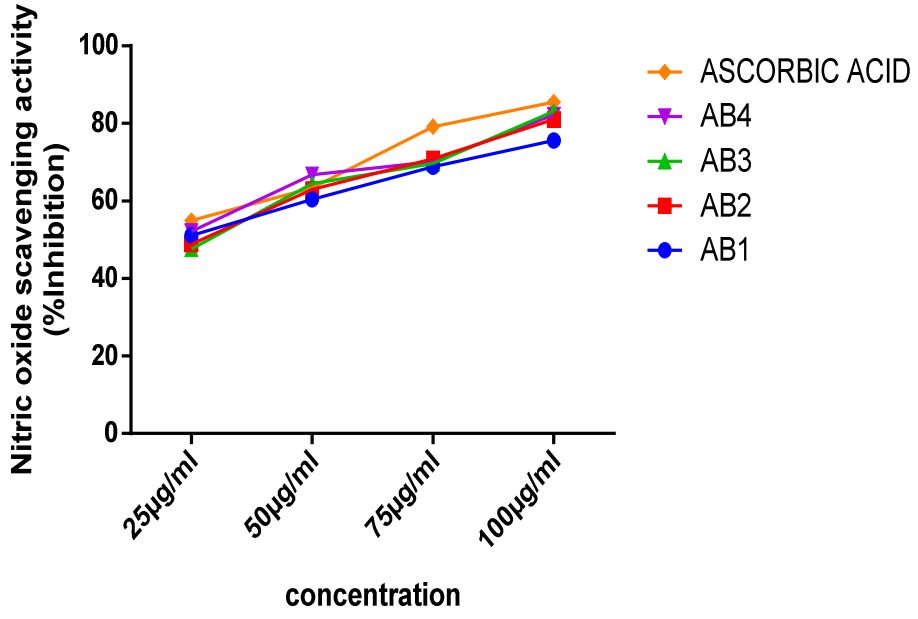

Fig. 5: Nitric Oxide scavenging activity of alcoholic beverages samples.

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 (Thupurani *et al.*, 2012). 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 50µg/ml, AB_4 had an inhibition 66.78% which is higher than ascorbic acid inhibition of 63.4%, clearly indicating that AB_4 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 (Hasan *et al.*, 2009) 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.

Reducing Power Property

From the results obtained in Figures 4, 4.3.2 and 4.3.3, at the least concentration of 25µg/ml, T_1 showed peak activity of inhibition whereas sample J_2 had peak activity over all other samples at concentrations 50µg/ml, 75µg/ml and 100µg/ml as determined against a reference antioxidant

Table 4: Percentage (%) reducing power

Sample	25µg/ml		50µg/ml		75µg/ml		100µg/ml	
T_1	0.134	±0.001	0.204	±0.001	0.377	±0.001	0.486	±0.002
T_2	0.147	±0.002	0.222	±0.002	0.412	±0.001	0.455	±0.003
C_1	0.126	±0.002	0.235	±0.001	0.391	±0.002	0.434	±0.003
C_2	0.127	±0.002	0.245	±0.002	0.366	±0.002	0.443	±0.001
J_1	0.141	±0.002	0.212	±0.002	0.337	±0.001	0.423	±0.001
J_2	0.126	±0.001	0.264	±0.002	0.423	±0.002	0.534	±0.001
J_3	0.146	±0.001	0.218	±0.002	0.293	±0.002	0.484	±0.001
J_4	0.124	±0.002	0.202	±0.001	0.267	±0.002	0.461	±0.002
AB_1	0.140	±0.002	0.214	±0.001	0.248	±0.001	0.375	±0.001
AB_2	0.118	±0.001	0.167	±0.002	0.237	±0.002	0.384	±0.002
AB_3	0.105	±0.001	0.145	±0.003	0.265	±0.003	0.387	±0.003
AB_4	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

v*alues are expressed as mean ±S*J_1 = apple juice, AB_1 = Herbal bitters, T_1 = green tea, J_2 = grape juice, AB_2 = Dry gin, T_2 = black tea, J_3 = orange juice, AB_3 = Brandy, J_4 = mango juice, AB_4 = Red wine, C = instant coffee

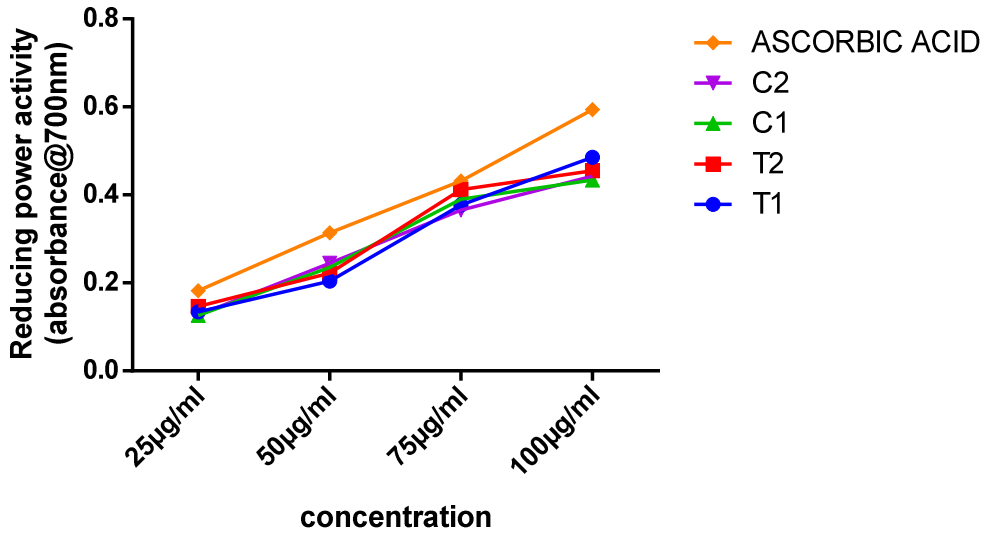

Fig. 4.3.1 Reducing power activity of coffee and tea

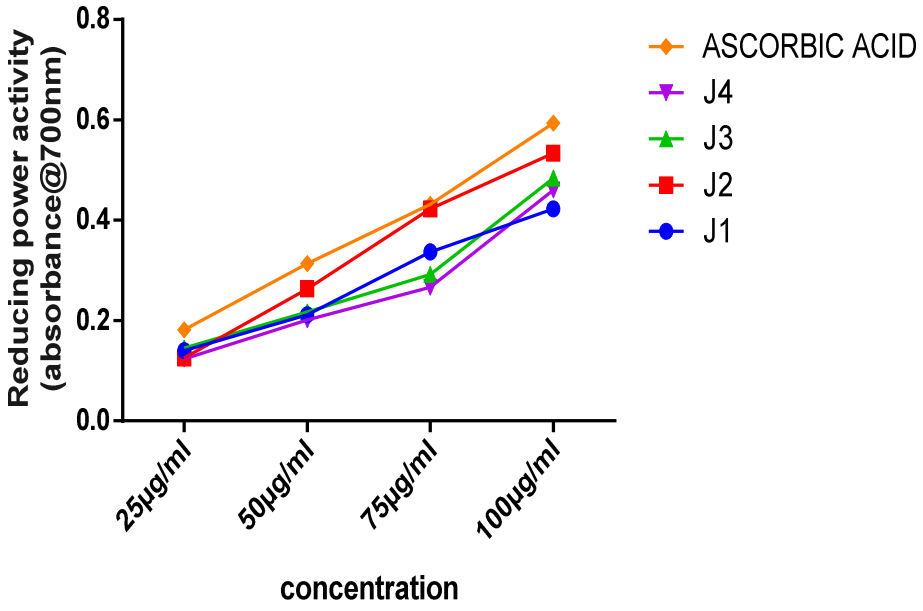

Fig. 4.3.2 Reducing power activity of juice samples.

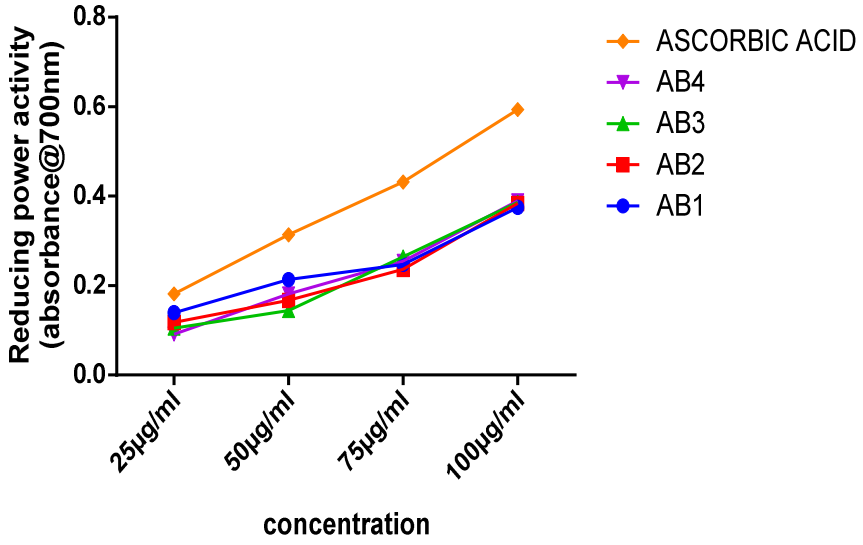

Fig. 4.3.3 Reducing power activity of alcoholic beverage samples.

Reducing power is a novel antioxidation defense mechanism; the two mechanisms that are available to affect this property are electron transfer and hydrogen atom transfer (Dastmalchi *et al.,* 2007).The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity (Koedel *et al.*, 2002), 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 (Zha *et al.*, 2009). Samples showed varying reducing capacity with respect to concentration following the trend that was set in the DPPH scavenging assay. Also, J_2 had the best antioxidant property with regards to reducing power because of its consistency of highest values (0.264mgAAE/100g, 0.423mgAAE/100g and 0.534mgAAE/100g) at the respective concentrations of 50µg/ml, 75µg/ml and 100µg/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 (Belitz *et al.*, 2009).

Total Antioxidant Capacity

Table 4.5 shows the results of total antioxidant capacity assay for each sample. From the table, J_2 has the highest value for total antioxidant capacity compared to AB_2 which has the least total antioxidant capacity value.

Table 4. Total Antioxidant Capacity (mg/100g) of samples.

	T_1	T_2	C_1	C_2	J_1	J_2	J_3	J_4	AB_1	AB_2	AB_3	AB_4
Mean	37.91	39.67	42.24	55.76	70.18	78.68	29.83	52.86	28.11	17.41	49.02	21.78
Std. Deviation	±0.3465	±0.1768	±0.5798	±0.2828	±0.2263	±0.2333	±0.3465	±0.3465	±0.2333	±0.3465	±0.2263	±0.1768

Values represented as mean ± standard deviation (N=2)

Key:

J_1 = apple juice
J_2 = grape juice
J_3 = orange juice
J_4 = mango juice
C = instant coffee
AB_1 = Herbal bitters
AB_2 = Dry gin
AB_3 = Brandy
AB_4 = Red wine
T_1 = green tea
T_2 = black tea

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 (Prieto *et al.,* 1999).

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 carotenoids, flavonoids, and cinnamic acid derivatives (Prieto *et al.,* 1999).

Total phenolic content

Table 4.1 shows an estimation of the total phenolic content of the samples. It was observed that C_2 and J_1 have the highest and lowest total phenolic content respectively.

Table 4.4. Total phenolic content (mg/g) of samples

	T_1	T_2	C_1	C_2	J_1	J_2	J_3	J_4	AB_1	AB_2	AB_3	AB_4
Mean	49.93	61.24	41.22	50.59	19.09	44.62	25.41	26.74	29.71	25.02	34.12	28.97
Std. Deviation	±0.2758	±0.3818	±0.3323	±0.2192	±0.3889	±0.1626	±0.2758	±0.1626	±0.2758	±0.3818	±0.3323	±0.3323

Values represented as mean ± standard deviation (N=2)

Key:

J_1 = apple juice
AB_1 = Herbal bitters
T_1 = green tea
C = instant coffee
J_2 = grape juice
AB_2 = Dry gin
T_2 = black tea
J_3 = orange juice
AB_3 = Brandy
J_4 = mango juice
AB_4 = Red wine

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 (Dumbravă *et al.*, 2011). Phenolic phytochemicals inhibit autoxidation of unsaturated lipids, thus preventing the formation of oxidized low-density lipoprotein (LDL), which is considered to induce cardiovascular disease (Amic *et al.*, 2003). The total phenolic content of the samples as determined only gives the quantity of polyphenols and phenolic compounds present in the samples without identifying the specific types and their individual quantities.

From Table 4.4, 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. T_2 was also shown to have a higher phenolic content that is statistically significant compared to T_1. This is contrary to the findings of Li *et al.,* (2011), Lubomila *et al.,* (2004), Oboh and Omoregie (2011) that green tea had higher phenolic content compared to black tea. The total phenolic content recorded for AB_3 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 (Charles, 2005).

5. 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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