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Total Phenolics and Antioxidant Capacity of Some Nigerian Beverages

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ABSTRACT: The aqueous extract of Nigerian beverages namely: fortified cocoa powder (Samples; FC_A , FC_B ,FC_C ,FC_D) Pure cocoa powder (PC_A, PC_B), coffee (C), ginger (G) and Tea samples (Green, TA, TB) were assayed for total phenols, flavonoids, Vitamin C and radical scavenging abilities using four different *in vitro* antioxidant assay methods. Coffee contains the highest amount of total phenols (135.71±0.92mg/g) and Vitamin C. (62.90± 2.97mg/g). The highest amounts of flavonoids were found in all the tea samples (10.0± 0.00mg/g). Green tea and coffee had the highest 2, 2-diphenyl-1-picrylhydrazyl radical [DPPH] radical scavenging ability. Green tea also had the highest radical scavenging ability as measured by the 2, 2-azino-di-[3-ethylbenzothialozine-sulphonic acid] [ABTS] radical scavenging ability and ferric reducing antioxidant property [FRAP]. (1.04± 0.037mg/g and 1235.88 ± 22.67mmol/g respectively). Cocoa powder (PC_A) had the highest iron II chelating ability (38.46 ± 2.72mg/g). The total phenolic content of the beverages had good correlation with total flavonoids, DPPH, ABTS and FRAP radical scavenging abilities with r values higher than 0.7. Vitamin C assay correlated well with DPPH free radical scavenging ability assay (r = 0.73) while iron (II) chelation ability correlated fairly with vitamin C (r = 0.43). The results suggest that the beverages possess significant radical scavenging ability that may be due to the presence of the antioxidant.
Keywords: Phenolics, flavonoids, Vitamin C, radical scavenging ,antioxidant Green tea and coffee .

INTRODUCTION

Excessive accumulation of free radicals results in oxidative stress. This is one of the main causes of initiation and progression of diseases and premature aging (Yasin *et al.,* 2010). The antioxidant system in healthy humans functions to scavenge free radicals and turn them to harmless particles (Silva *et al.,* 2006). Adverse factors (stress, diseases, certain powerful drugs, environmental pollution, smoking, alcoholism, low quality food products, etc) increase free radical generation which could damage DNA, proteins, lipids carbohydrates and vascular walls (Sun *et al.,* 2002). These could result in disorganization of normal processes in human bodies (Gutteridge and Halliwell, 2000).

Increased antioxidant intake may protect humans from diseases as oxidative events in vivo may play a role in the pathogenesis of many diseases which includes cancer, cardiovascular diseases and arthritis (Oboh and Rocha, 2007, Ghanim *et al.,* 2010).

Plant foods rich in antioxidants have received a growing interest because they delay oxidative stress (Nicolle *et al.,* 2004).The chemoprotective properties of phenols and flavonoids have been reported to include antithrombotic, pharmacological and hypolipidemic effects. (Dreosti, 2000).Vitamin C may also contribute to the maintenance of a healthy vasculature and a reduction in atherogenesis through regulation of collagen synthesis, prostacyclin production and nitric oxide (Aruoma, 2003).

Beverages (Coffee, tea and cocoa) contain polyphenols and flavonoids that have diverse beneficial biochemical and antioxidant effects (Buijsse *et al.,* 2006; Schroeter *et al;* 2002). These beverages may play an important role in improving beneficial intestinal microflora, as well as providing immunity against intestinal disorders and in protecting cell membranes from oxidative damage (Ferrazzano *et al.,* 2009).

The antioxidant effects of some beverages consumed has been reported (Pellegrini *et al.,* 2003; Rawell and Kulling; 2007).

This study aims to determine the total phenol, flavonoid and vitamin C contents in some Nigerian beverages and assess the free radical scavenging abilities of the aqueous extracts using four different *in vitro* assays.

MATERIALS AND METHODS

Materials

Samples of beverages namely: Fortified Cocoa powder (FC_A, FC_B, FC_C, FC_D), pure cocoa powders (PC_A, PC_B), coffee (C), ginger (G), teas: (Green, T_A, T_B), were purchased at both the Oba market and New Benin market in Benin metropolis, Nigeria.

Preparation of Aqueous Extract

The aqueous extract of the different beverages were prepared by adding 100ml of boiling water to 1g, stirred and left for five minutes. Thereafter, the mixtures were centrifuged at 500rpm for 5minutes. The supernatant (aqueous extract) was collected and stored at 0^0C until needed for analysis.

METHODS

Evaluation of Antioxidant Activity

A. 1, 1-diphenyl–2 picrylhydrazyl free radical scavenging ability [DPPH]

The free radical scavenging ability of the extracts against DPPH (1, 1-diphenylï 2 picrylhydrazyl) free radical was evaluated as described by Gyamfi *et al.* (1999). A 1:10 dilution of the extracts was prepared and 1 ml of it was mixed with 1 ml, 0.4 mM methanolic solution containing DPPH radicals. The mixture was left in the dark for 30 min and the absorbance taken at 516 nm using UV-Visible spectrophotometer, JENWAY, GERMANY. The DPPH free radical scavenging ability was subsequently calculated.

B. 2, 2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) [ABTS] Radical Scavenging Ability

The ABTS scavenging ability of the extracts was determined according to the method described by Re *et al.* (1999). The ABTS was generated by reacting ABTS aqueous solution (7 mmol/l) with $K_2S_2O_8$ (2.45mmol/l, final concentration) in the dark for 16 h and adjusting the Absorbance at 734nm to 0.700 with ethanol. The fortified cocoa powders and ginger drink were undiluted, while a 1:10 dilution of the other beverages was utilized for the assay. An aliquot of 0.2ml from each extract was then added to 2.0ml ABTS solution and the absorbance were measured at 734nm after 15mins using the UV-Visible spectrophotometer, JENWAY, GERMANY. The trolox equivalent antioxidant capacity was subsequently calculated.

C. Ferric Ion Reducing Antioxidant Power (FRAP) assay.

The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$ solution as described by Oyaizu (1986). A 2.5ml aliquot of each extract was mixed with 2.5 ml 200 mM sodium phosphate buffer (pH 6.6) and 2.5 ml 1% potassium ferricyanide. The mixture was incubated at 50^oC for 20 min. and then 2.5 ml 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. The supernatant were collected and 5ml aliquot of each extract was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm. The ferric ion reducing antioxidant property was then calculated.

D. Iron (Fe^{2+}) Chelation Assay

The Fe^{2+} chelating ability of the extracts was determined using a modified method of Minotti and Aust (1987) with a slight modification by Puntel *et al.,* (2005). Freshly prepared 500µM $FeSO_4$ (150 µl) was added to a reaction mixture containing 168 µl 0.1M Tris-HCl (pH 7.4), 218 µl saline and 100 µl was taken from the 1:10 dilution of the extracts. The reaction mixture was incubated for 5min, before the addition of 13 µl 0.25% 1, 10-phenanthroline (w/v). The absorbance was subsequently measured at 510 nm. The Fe (II) chelating ability was then calculated

E. Determination of Total Phenol Content

The total phenol content was determined according to the method of Singleton *et al.* (1999). A 100 µl of the 1:10 dilution of the aqueous extracts was oxidized with 2.5ml 10% Folin-Ciocalteauûs reagent (v/v) and neutralized with 2.0ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40minute at 45^oC and the absorbance was measured at 765nm. The total

phenol content was subsequently calculated as gallic acid (10mg/100ml) equivalent.

F. Determination of Total Flavonoid Content

The total flavonoid content was determined using a slightly modified method of Meda *et al.* (2005), 0.5ml of 1:2 diluted sample was mixed with 0.5ml methanol, 50µl 10% $AlCl_3$, 50µl 1M Potassium acetate and 1.4ml water, and allowed to incubate at room temperature for 30min. The absorbance of the reaction mixture was then measured at 415 nm. The total flavonoid content was subsequently calculated using quercetin (10mg/100ml) as standard.

G. Determination of Vitamin C

Vitamin C content of the aqueous extract was determined using the method of Benderitter *et al.,* (1998). DNPH [2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg CuSO4.5H2O in 100ml of 5 M H_2SO_4] was prepared and 75 εl of it was added to 500 εl reaction mixtures [300εl of the aqueous extracts and 100 εl of 13.3 % trichloroacetic acid (TCA)]. The reaction mixture was subsequently incubated for 3 h at 37^{o}C, then 0.5 ml of 65 % H_2SO4 (v/v) was added to the medium and the absorbance was measured at 520 nm. The vitamin C content of the extracts was subsequently calculated using ascorbic acid (10mg/100ml) as standard.

Statistical Analysis

All experiments were carried out in duplicates. Results are expressed as mean ± standard error of the means. Pearsonôs correlation coefficient were calculated from the results using Microsoft Excel 2003.The results were statistically analyzed by Analysis of variance (ANOVA), and Duncan new multiple range tests. Statistical significance was accepted at PÒ0.05.

RESULTS

Total phenols, Flavonoids and Vitamin C content of the Beverages.

The results for the total phenols, flavonoid and vitamin C contents of the beverages are shown in Table 1. The results reveal that ginger drink had the lowest phenol content (5.15 ± 0.07mg/g), while coffee had the highest (135.7 ± 0.92mg/g). Ginger drink and the fortified cocoa powders (FC_A, FC_B, FC_C, FC_D) had the lowest flavonoid content (3.33mg/g each), while the teas (Green tea, T_A and T_B tea samples) had the highest (10.00mg/g).

The ginger drink and the fortified cocoa powders (FC_A, FC_C, FC_D) were not significantly different at P > 0.05 in their phenol and flavonoid content. The total phenol content in green tea and tea sample A, showed no significant difference at P > 0.05 while coffee and tea sample B were significantly (P Ò 0.05) higher than the other beverages. Coffee had the highest value of vitamin C (62.90mg/g), while ginger drink had the lowest value of 1.20mg/g. The pure cocoa powders were significantly (P Ò 0.05) higher in vitamin C than the fortified cocoa samples.

Antioxidant activity of the Beverages

The results for the evaluation of antioxidant activity of the Nigerian beverages as assessed by four *in vitro* methods (DPPH, ABTS radical scavenging ability, ferric reducing antioxidant property and Iron (II) chelating ability) are shown in Table 2.

The DPPH radical scavenging activity determination showed that green tea had the highest ability (68.90%) while ginger drink had the lowest scavenging ability (8.43%). The means of DPPH scavenging activity followed the descending order: green tea > tea sample A > coffee >tea sample B > pure cocoa powder A > pure cocoa powder B >fortified cocoa powder $FC_D > FC_B > FC_C > FC_A$ >ginger drink.

The ABTS scavenging ability and ferric reducing antioxidant property (FRAP) revealed that green tea had the highest scavenging ability and reducing property of 1.048mmol/g and1235.88mg/g respectively while ginger drink had the lowest value of 0.014mmol/g and 44.28mg/g respectively. The ABTS and FRAP antioxidant activity assay showed a similar trend with that observed for the DPPH assay.

The ferrous ion chelating abilities of the beverage studied showed that the pure cocoa powder (PC_A and PC_B) had the highest chelating ability of 43.27mg/g and 38.46mg/g respectively. The fortified cocoa powder FC_A , had the lowest ability of 3.85mg/g. The fortified cocoa powder FC_A and FC_B ; and tea sample T_A were

significantly different at (P Ò 0.05) .Coffee, ginger drink, green tea and tea sample B had ferrous ion chelating abilities that were not significantly different at (P Ò0.05).

Table 1: Phenolic Distributions (Total Phenol and Flavonoids) and Vitamin C in the Beverages**.**

Beverage Samples	Total Phenol (mg/g)	Total Flavonoids (mg/g)	Vitamin C (mg/g)
Fortified cocoa beverage			
FC_A	5.19 ± 1.84^a	3.33 ± 0.00^c	9.15 ± 1.20^g
FC_B	13.64 ± 2.71^b	3.33 ± 0.00^c	29.15 ± 1.20^d
FC_C	5.84 ± 0.92^e	3.33 ± 0.00^c	9.15 ± 2.33^g
FC_D	9.09 ± 1.27^e	3.33 ± 0.00^c	24.60 ± 0.57^e
Pure cocoa powder			
PC_A	41.56 ± 2.71^d	5.00 ± 2 .36^b	49.60 ± 0.57^c
PC_B	32.47 ± 9.18^d	5.00 ± 2.36^b	48.75 ± 0.64^c
Coffee	135.71 ± 0.92^a	6.67 ±0.00^b	62.90 ± 2.97^a
Ginger Drink	5.15 ± 0.07^e	3.33 ± 0.00^c	1.20 ± 0.56^h
Tea			
Green tea	99.90 ± 1.69^b	10.00 ± 0.00^a	13.35 ± 1.20^f
T_A	95.521 ±.01^b	10.00 ± 0.00^a	57.05 ± 1.77^b
T_B	70.12 ± 5.51^c	10.00 ± 0.00^a	47.50 ± 3.53^c

Values are means ± standard error of the mean (SEM).
Means having different superscript along the same column are significantly different at P Ò0.05.

Table 2: DPPH free radical scavenging ability, ABTS scavenging ability, ferric reducing antioxidant properties (FRAP) and Fe (II) chelating ability of the beverages.

Beverage Samples	DPPH (%)	ABTS (mmol/g)	FRAP (mg/g)	Fe^{2+} Chelation (mg/g)
Fortified cocoa powder				
FC_A	12.21 ± 1.64^d	0.028 ± 0.000^f	66.41 ± 20.51^f	3.85 ± 0.00^e
FC_B	24.24 ± 4.37^c	0.022 ± 0.001^f	154.20 ± 8.64^e	22.12 ± 4.08^b
FC_C	21.52 ± 3.29^c	0.028 ± 0.000^f	105.34 ± 10.50^e	17.31 ± 2.72^c
FC_D	26.75 ± 9.86^c	0.023 ± 0.001^f	123.66 ± 21.59^e	17.31 ± 2.72^c
Pure cocoa powder				
PC_A	45.93 ± 0.00^b	0.280 ± 0.011^e	583.20 ± 77.73^c	38.46 ± 2.72^a
PC_B	55.52 ± 6.71^b	0.238 ± 0.011^e	407.64 ± 53.97^d	43.27 ± 4.08^a
Coffee(Nescafe)	64.83 ± 2.06^a	0.891 ±0.040^c	1063.36 ±134.94^b	21.15 ± 8.16^b
Ginger Drink	8.43 ± 2.51^d	0.014 ± 0.001^g	44.28 ± 2.16^f	18.27 ± 4.08^c
Tea				
Green tea	68.90 ± 2.06^a	1.048 ± 0.037^a	1235.88±22.67^a	17.30 ± 0.00^c
T_A	62.80 ± 3.29^a	0.995 ± 0.042^b	1066.41±87.44^b	12.50 ± 1.36^d
T_B	59.89 ± 0.83^{ab}	0.761 ± 0.004^d	687.02 ± 41.02^c	17.31 ± 2.72^c

Values are means ± standard error of the mean (SEM).
Means having different superscript along the same column are significantly different at P< 0.05

DISCUSSION

Coffee, tea and cocoa powders are products that are increasingly used as functional beverages in Nigeria. The potential health benefits that are ascribed to them are due to the bioavailability of the antioxidant compounds they contain (Iwalewa *et al*., 2005).

The high phenol content in coffee could be due to the starting materials, roasting levels and brewing method (Ferruzi, 2010).

Processing of raw cocoa beans involves fermentation, drying, and roasting (Wood and Las, 2001). This alters its chemistry. During cocoa fermentation, polyphenols are subjected to biochemical modifications through oxidation, polymerization and binding with proteins (Nazaruddin *et al*. 2006a).This leads to decreased solubility and astringency effects (Drying reduces the amount of polyphenols substantially by enzymatic browning (Kyi *et al*. 2005). On the contrary, the roasting process, which is responsible for reducing bitter and acidic tastes, causes small changes in polyphenolic concentration (Misnawi *et al*. 2002; Nazaruddin *et al*. 2006b). The significantly (PÓ 0.05) higher phenolic contents of the pure cocoa powders (PC_A,PC_B) than the fortified cocoa powders (FC_A,FC_B,FC_C,FC_D) could be due to fermentation of the fortified cocoa powder during preparation.

Green tea had the highest phenolic and flavonoid contents of the teas studied. This may be due to the fact that in its processing, it is neither wilted nor oxidized (Graham, 2006). Wilting and oxidation cause changes in polyphenol content (Cabrera *et al.,* 2006). Green tea has 30-40% epigallocatechingallate (EGCG). This result is in agreement with the earlier reported that teas contains more than 700 chemicals, among which the compounds closely related to human health are flavonoids, amino acids, vitamins C, K and E, caffeine and polysaccharides. It also agrees with reports where correlations were established between total phenolic and total flavonoid contents (Melo *et al.,* 2006). Green tea had the highest DPPH scavenging ability.

DPPH is frequently used in the determination of free radical scavenging ability; however, it has the limitation of colour interference and sample solubility. Therefore, the free radical scavenging ability of the plant extracts were further studied using a moderately stable nitrogen-centered radical species: ABTS (2,2-azinobis (3-ethylbenzo-thiazoline- 6-sulfonate). The ABTS radical based model of free radical scavenging ability has the advantage of being more versatile as both non-polar and polar samples can be assessed and spectral interference is minimized as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products (Re *et al.,* 1999). ABTS and DPPH free radical scavenging abilities follow the same trend. This was found to be; ginger drink< fortified cocoa powders <pure cocoa powders< tea sample B< coffee< tea sample A < green tea in ascending order.

The techniques used for determining antioxidant activity, DPPH and ABTS free radical scavenging ability and FRAP had good positive correlations with the total phenol and flavonoid content of the beverages. (Table 3).This indicates that phenolic compounds are important contributors to antioxidant activity of the beverages. Similar results have been reported (Aikpokpodion and Dongo, 2010). It could be that phenolic compounds (where flavonoids is one of the main class), are known to be hydrophilic antioxidants and are the most abundant secondary metabolite in plants (Gil *et al.,* 2002). Furthermore, good correlations were obtained from DPPH and ABTS free radical scavenging ability and FRAP indicating that the beverages had comparable activities in the three assays. High correlation is also found between these three assays in other plant derived samples (Connor *et al.,* 2002).

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 powers of the beverage extracts were assessed based on their ability to reduce Fe(III) to Fe(II). The result follows the same trend as ABTS and DPPH free radical scavenging ability. This goes on to confirm that there is a correlation between total phenol contents and overall antioxidant capacity in foods. Neurodegenerative diseases and aging processes are associated with iron accumulation and this condition could be prevented by Fe chelators (Fraga and Oteiza, 2002). The ability of antioxidants to chelate and deactivate transition metals prevents such metals from participating in the initiation of lipid peroxidation and oxidative stress through metal-catalyzed reaction (Dastmalchi *et al.,* 2007). The ability of the extracts to chelate transition metals is therefore considered to be due to an antioxidant mechanism (Oboh *et al.,* 2007). The results revealed that all the extracts chelate Fe (II). Pure cocoa powders

which were the highest chelators of iron, while the fortified cocoa powder (FC_A) was the lowest chelator .The trend of this result is slightly similar with that of vitamin C distribution among the beverages. This similarity between the vitamin C content and the iron(II) chelating ability may confirm that vitamin C chelates heavy metals, reduces free radicals and suppress perioxidation thereby reducing the risk of artheriosclerosis, cardiovascular diseases and some cancers (Temple, 2000).

The low values for total phenol, flavonoid, Vitamin C and three antioxidant scavenging abilities (DPPH,ABTS and FRAP) of ginger tea, observed in this study, show that ginger tea exhibits low free radical scavenging abilities. Vitamin C has been reported to contribute to the antioxidant activities of plant food. Ascorbic acid is a good reducing agent and exhibits its antioxidant activities by electron donation (Oboh and Akindahunsi, 2004).

Table 3: Correlation coefficients of Total Phenol, Total Flavonoids, Vitamin C, Ferric Reducing Antioxidant Property (FRAP), ABTS Scavenging Ability, DPPH Free Radical Scavenging and Iron (II) Chelation Abilty**.**

	Total Phenol	Total Flavonoid	Vitamin C	FRAP	ABTS Scavenging ability	DDPH Free Radical Scavenging	Iron (II) Chelation Ability
Total Phenol	1						
Total Flavonoid	0.818345	1					
Vitamin C	0.647671	0.476165	1				
FRAP	0.95985	0.886159	0.578889	1			
ABTS Scavenging ability	0.95435	0.945882	0.539173	0.975472	1		
DDPH Free Radical Scavenging	0.881724	0.85695	0.730549	0.925497	0.897748	1	
Iron (II) Chelation ability	-0.01306	-0.10482	0.43899	0.047988	-0.09632	0.294972	1

CONCLUSION

The study revealed that; Green tea had the highest antioxidant activity, while coffee had good antioxidant capacity. Among the cocoa beverage, the pure cocoa powders (PC_A and PC_B) had more antioxidant capacity than the fortified cocoa powders (FC_A , FC_B ,FC_C ,FC_D). Ginger drink may not be a suitable beverage to provide antioxidants as it had the lowest antioxidant activity of all the beverages.

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