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Antioxidant and Free Radical Scavenging Abilities of some Indigenous Nigerian Drinks

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ABSTRACT: Antioxidant contents of five local Nigerian drinks namely: 'Kunu', palmwine, plantain, soyabean and 'zobo' drinks were analyzed for flavonoids, phenols and vitamin C. The antioxidant scavenging abilities were evaluated using four invitro methods. 'Zobo' drink with sugar (Zs) had the highest phenolic contents (16.00 ±0.26mg/ml) while 'zobo' drink with ginger (Z_G) recorded the highest flavonoids and vitamin C (3.91±0.02mg/ml and 2.31±0.01mg/ml) respectively. Local palm wine had the low
est phenols, flavonoids and vitamin C contents. (1.44± 0.00mg/ml, 0.10±0.04mg/ml and 0.29±0.02mg/ml respectively. 'Zobo' drink without sugar (Z_{NS}) had the highest 2,2-diphenyl-1-picrylhydrazyl (DPPH) and the nitric oxide (NO) scavenging abilities of (70.18±0.65 % and 52.63±0.00%) respectively. Z_G had the highest ferric reducing antioxidant property (FRAP), of 32.43±0.19 mgGAE/ml . A strong positive correlation exists between the total flavonoid, phenol and vitamin C content with their antioxidant capacities. Soybean was second to 'zobo' drinks in the scavenging of the DPPH radical and ferric reducing ability. 'Kunu' drinks had the lowest ability to mop up the DPPH radical (12.85±0.00), but a fair ability in the mopping up of the ABTS (0.32±0.001) and NO (21.05±7.44) radicals.. Plantain drink showed significantly ($P \leq 0.05$) lower levels of flavonoids and vitamin C in comparison to kunu drink.
Keywords: Phenolics, flavonoids, Vitamin C, radical scavenging ability.

INTRODUCTION

Local indigenous Nigerian drinks are becoming popular in urban cities because they are cheaper than imported drinks. These drinks are well accepted among Nigerians and they make positive contributions to the agricultural and marketing sectors of the country.

'Kunu' is a cereal based non-alcoholic fermented beverage. The cereals used in its production are millet, sorghum and maize which are in decreasing order of preference (Gaffa *et al*., 2002). It has low viscosity, with sour-sweet taste and is used as weaning drinks for infants in some communities (Sowonola *et al*., 2005). The nutritional and sensory quality characteristic of kunu has been reported (Akoma *et al.,* 2002). It has a milky appearance and it is common with the northern people of Nigeria (Adeyemi and Umar, 1994). It is generally consumed on its own by adult as a thirst quencher and serve as refreshment in some communities.

Palm wine drink is made from the sap of the raffia palm which ferments within few hours and turns to an alcoholic drink. The nutrient composition of the fresh sap has been reported (Obahiagbon and Osagie, 2007). The palm drink is a rich nutrient medium containing sugars, protein, amino acids, alcohol and minerals (Ezeagu *et al*., 2003).

Plantain drink is a fermented beverage produced by fermentation of ripe plantain. Plantain (*Musa paradisciaca L*) is a boat shaped fruit produced by the shrub. It is cultivated in Nigeria as a source of starchy staple (Adeniyi *et al*., 2006). The mature unripe finger is green in color but changes to a yellow color on ripening. The proximate content, nutrient composition, functional characteristics and properties of ripe and unripe plantains have been evaluated (Adegboyega, 2006; Izunfuo and Omuaru, 2006; Mohammed and Saleha, 2011).The plantain drink is a whitish effervescent liquid with a sweet taste (Idabacha and Onyezili, 1994).

Soybean milk drink is produced from soybean (*Glycine max*) seeds, by grinding and filtration. Soybeans are legumes that contain no cholesterol and are low in saturated fat (Lindsay and Claywell, 1998). They are also the only vegetable food that contain all the eight essential amino acids (Morrison and Hark, 1999; Dudek, 2001). Soybean is also rich in phytochemicals that are beneficial to the human being and so, it is considered as a nutraceutical or a functional food crop (Babu Shankar Ponnusha *et al*., 2011). These phytochemicals include soy polyphenols, particularly the isoflavones and anthocyanins (Mebrahtu *et al.,* 2004). They have been reported to have antioxidant, antiproliferative and hypocholesterolemic effects.(Messina, 2000,

Isanga and Zhang, 2008). Recent studies have shown that soybean protein contributes to the control of hyperglycemia and reduces body weight, hyperlipidemia and hyper insulinemia (Bhathena and Velarquez, 2002).

Zobo drink is a non alcoholic local beverage made from different varieties of dried petals and calyces of the flower *Hibiscus sabdariffa* by boiling and filtration (Kolawole and Okeniyi, 2007; Ogiehor *et al*., 2008). The calyces of *H. sabdariffa* are rich in vitamins, natural carbohydrate, protein and vitamin C with other antioxidants (Wong *et al*., 2002) and minerals (Babalola *et al*., 2000). *H. sabdariffa* L is also used in folk medicine against many complaints that include high blood pressure, liver diseases and fever (Dalziel, 1973; Wang *et al*., 2000; Odigie *et al*., 2003; Ali *et al*., 2005).

This study aims to evaluate the antioxidant and in vitro free radical scavenging abilities of these indigenous drinks.

MATERIALS AND METHODS

Materials

The raw materials for the preparation of the drinks, Soya beans, *Hibiscus spp* calyces, millet grains and ripe plantain were purchased from New Benin market, Benin City, Edo State, Nigeria. Raphia palm wine was purchased from the Nigeria Institute for Oil Palm Research (NIFOR) in Edo State, Nigeria. The local palm wine was purchased from a local palm wine tapper in Benin City, in Edo State, Nigeria.

Methods

Preparation of the Drinks

'Kunu' Drink

'Kunu' drink was prepared from millet (*Pennisetum typhoides)* Obadina *et al*., (2008). The millet grain was sorted and cleaned to remove foreign materials. 600g of millet grains were soaked in 1.2 dm^3 of water (1:2 w/v) for 24 h (Adeyemi and Umar, 1994).

The grains were then wet-milled to almost smooth slurry and filtered through muslin cloth. The sediment was re-extracted with hot water. The filtrate was then allowed to stand for 2 hours for the starch fraction to settle while the top liquid was decanted.

The starch fraction after weighing was 230g and was then divided into two parts (3:2). The larger proportion weighed 140g was cooked with 600ml of boiling water for 5 minutes while the smaller fraction, 90g was boiled with cold water (600ml) for 5 minutes while stirring. The total extracts were collected. Two different portions made. One portion was sweetened with table sugar (100% sugar w/w millet seeds).The other was not sweetened.

Palm wine

Bottled Raphia wine was obtained from the commercial lot at NIFOR. This had been pasteurized and bottled under approved clinical conditions. The drink was purchased from a local palm wine tapper and stored in containers in the refrigerator about 0^oC (which is the usual practice among palm wine (Dealers).

Plantain Drink

Ripe plantain (500g) was peeled, cut into smaller pieces and steeped in 1500ml of cold water, (1:3 w/v) for 48 hours (Ohiokpakai, 1985). The sample was then filtered with a fine cloth (sterile) while the slurry was discarded. The liquid was then bottled.

Soyabean Drink

Soybean seeds (200g) were sorted out to remove dirt and broken grains, washed and soaked in approximately 600ml of water (1:2 w/v) until they have about doubled their weight for 24 h. The soaked soybean seeds were added to 800ml of water to blend (1:8 w/v). The soluble soybean emulsion was readily separated from the insoluble residue by passing the ground slurry through a fine cloth filter. The slurry was then discarded and the milk boiled for 30 minutes. Two different portions were made. One portion was sweetened with table sugar (5% w/v) added to the soymilk. The other was not sweetened.

' Zobo' Drink

Roselle calyces (46g) were steeped into 460mls of water (80^oC for 30 minutes) and filtered using a clean sterile sieve cloth. (Abdullahi and Elegbe, 2001; Onwuka and Omeire 2001; Fasoyinso *et al.,* 2005).The filtrate was then sweetened with 46g of sugar (1:10 w/v). The liquid was not pasteurized. The drink was divided into three equal portions. One portion had sugar, another without sugar. The third portion was prepared with ginger. Ginger (5g) was grated and added to water (50ml).This was boiled for 10 minutes, and added to the third portion.

Statistical Analysis

All data were expressed as means ± standard deviation of duplicate determinations. Data were analyzed by ANOVA (analysis of variance) and DUNCAN multiple range test was used to assess the difference between groups while correlation study was used to assess the relationship between the

antioxidants and antioxidant assays. A significant difference was considered at the level of P≥0.05.

RESULTS

Total Phenol, Flavonoid and Vitamin C

The total phenol, flavonoid and vitamin C content of the drinks are presented in Table 1. The phenolic content was highest in zobo drink without sugar (Z_{NS}) with a value of 16.81±0.26mg/ml gallic acid equivalent. The drink with the lowest phenolic content is the local palm wine with a value of 1.44±0.08mg/ml GAE. The result reveals that the different zobo drinks have significantly different (p≤0.05) phenol levels. Soybean milk and kunu drinks were not significantly different in phenolic contents (p≥0.05). Plantain drink and palm wine had no significantly different (p≥0.05) phenol levels.

The total flavonoid content was highest in zobo drink with ginger (Z_G) with a value of 3.91±0.22mg/ml. The other zobo drinks (Z_S/Z_{NS}) did not differ among the group. Local palm wine had the lowest flavonoid content (0.10±0.04mg/ml). Soybean milk and kunu drinks have no significant difference (p≥0.05). Palm wine had a significantly (p≤0.05) higher flavonoid value than plantain drink.

Zobo with ginger (Z_G) had the highest vitamin C content (2.31±0.01mg/ml of ascorbic acid equivalent). While the local palm wine had the lowest amount of vitamin C (0.29±0.02mg/ml AAE). Zobo drinks (Z_s/Z_{NS}) did not have any significant difference in the vitamin C levels. However Soybean milk drink with sugar differed significantly from soybean without sugar in Vitamin. C levels. This trend was also observed for the kunu drinks (0.77±0.02 and 0.65±0.06 respectively).

Antioxidant Capacity of the Drinks

The ABTS, DPPH, FRAP and NO scavenging ability of the drinks are presented in Table 2. The result show that all 'zobo' drinks had the highest antioxidant scavenging capability as measured by ABTS, FRAP, DPPH and NO. The result also showed that there was no significant difference among the zobo drinks in all the assays. Soybean drink ranked closely after zobo for DPPH and FRAP. Soybean with sugar was significantly lower in ABTS scavenging ability than Soybean without sugar. The plantain drink, kunu and palm wine drinks had the lowest ability to scavenge ABTS radical. FRAP ability was lowest in the palm wines. NO was lowest in the soybean drinks.

Table 1: Total Phenol, Flavonoid and Vitamin C Content of Nigerian Drinks.

Sample drinks	Total Phenol (mgGAE/ml)	Total Flavonoid(mg/ml)	Vitamin C (mg/ml AAE)
Zobo(+ginger)	12.00±0.70^{c}	3.91±0.22^{e}	2.31±0.01^{h}
Zobo(+sugar)	14.18 ±0.79^{b}	3.57 ±0.24^{d}	1.93±0.09^{fg}
Zobo(+no sugar)	16.81 ±0.26^{a}	3.53 ±0.09^{d}	2.00±0.00^{g}
Soybean(+sugar)	4.56±0.09^{d}	0.83±0.04^{c}	1.86±0.05^{f}
Soybean(+no sugar)	4.88±0.00^{d}	1.06±0.09^{c}	1.53±0.01^{e}
Kunu(+sugar)	2.00±0.17^{e}	1.10±0.14^{c}	0.77±0.02^{d}
Kunu(+no sugar)	1.56±0.09^{e}	0.83±0.04^{c}	0.65±0.06^{c}
Plantain drink	2.13±0.35^{e}	0.20±0.00^{ab}	0.44±0.02^{b}
Raphia palm wine	1.75±0.00^{e}	0.40±0.09^{b}	0.35±0.04^{ab}
Local palm wine	1.44±0.08^{f}	0.10±0.04^{a}	0.29±0.02^{a}

Values are expressed as mean ± standard deviation of duplicate determination.
Means with same superscript on the same are not significantly different at P≥0.05.

Table 2: Antioxidants capacity of Nigerian Drinks

Sample drinks	ABTS(mmol/100ml)	DPPH (%)	FRAP(mgGAE/ml)	NO (%)
Zobo(+ginger)	0.55±0.007^{e}	65.13±2.59^{a}	32.43±0.19^{d}	50.00±11.15^{c}
Zobo(+ sugar)	0.54±0.000^{e}	68.35±11.03^{a}	27.85±4.64^{d}	47.37±0.00^{c}
Zobo(+no sugar)	0.55±0.002^{e}	70.18±0.65^{a}	28.57±0.80^{d}	52.63±0.00^{c}
Soybean(+ sugar)	0.28±0.002^{ab}	41.28±0.38^{b}	8.85±0.20^{c}	10.53±0.00^{a}
Soybean(+no sugar)	0.46±0.003^{d}	44.49±12.32^{b}	8.00±0.19^{c}	10.53±0.00^{a}
Kunu(+ sugar)	0.28±0.002^{ab}	16.51±0.00^{c}	4.25±0.60^{bc}	23.68±3.72^{b}
Kunu(+no sugar)	0.32±0.001^{c}	12.85±0.00^{c}	4.43±0.00^{bc}	21.05±7.44^{ab}
Plantain drink	0.28±0.000^{a}	41.28±5.19^{b}	7.35±0.40^{bc}	21.05±7.44^{ab}
Raphia palm wine	0.28±0.006^{ab}	48.62±0.00^{b}	0.14±0.00^{a}	15.79±0.00^{ab}
Local palm wine	0.29±0.004^{b}	40.82±5.83^{b}	2.64±1.11^{ab}	15.79±0.00^{a}

Values are expressed as mean ± standard deviation of duplicate determination.

Means with same superscript on the same column are not significantly different at P≥0.05.

Correlation Studies

The correlation result (Table 3) indicat that the antioxidants had a positive correlation with the antioxidant capacity assays. Total phenol had strong positive correlation with ABTS (r=0.921), DPPH (r=0.829), FRAP(r=0.952) and NO (r=0.893).Total flavonoid content also had strong positive correlation with ABTS (r=0.928), DPPH (r=0.716), FRAP (r=0.970) and NO (r=0.932).The strong positive correlations between the total flavonoid and phenol contents and the antioxidant capacities are similar to those in some Nigerian fruits.Vitamin C correlated positively with ABTS (r=0.812), DPPH (r=0.648), FRAP (r=0.856) and NO (r=0.628).

Table 3: Correlation table of the total phenol, total flavonoids, vitamin C, ferric reducing antioxidant property, ABTS radical scavenging, DPPH radical scavenging and nitric oxide radical scavenging assays.

	Total phenol	Total flavonoid	Vitamin C	Reducing power	ABTS radical scavenging	DPPH radical scavenging	NO radical scavenging
Total phenol	1						
Total flavonoid	0.947	1					
Vitamin C	0.833	0.844	1				
Reducing power	0.952	0.970	0.856	1			
ABTS radical scavenging	0.921	0.928	0.812	0.914	1		
DPPH radical scavenging	0.829	0.716	0.648	0.783	0.765	1	
NO radical scavenging	0.893	0.932	0.628	0.920	0.823	0.671	1

DISCUSSION

Zobo drinks had the highest antioxidant content as presented in Table 1 which has strong positive correlation with the antioxidant scavenging capacity of the drinks. The roselle calyces of the *Hibiscus sabdariffa* used in preparing zobo drink is rich in vitamin C and flavonoids (Wong *et al*., 2002).This is responsible for the high antioxidant content seen in the zobo drinks which correlates with the high ability of the drinks to scavenge the ABTS, DPPH and NO radicals as seen in Table 2. Furthermore, the ferric reducing ability of the drinks was highest in the zobo drink possibly due to its high phenolic or flavonoid content (r=0.952 and r=0.970 respectively). However, the total phenolic content was significantly different (p≤0.05) in all the "zobo" drinks. This shows that sugar and ginger (*Zingziber officinale*) affected the total phenol of the drinks. However, "zobo" with ginger had a significantly (p≤0.05) higher flavonoid and vitamin C content compared to the other "zobo" drinks. Ginger is used as a spice and as natural additives for more than 2000 years (Bartley and Jacobs, 2000). Ginger has many medicinal properties and has been reported to be a good source of antioxidants which exhibit high activities (Shirin and Prakash, 2010). The strong positive correlation between the total flavonoid, phenol and vitamin C content with their antioxidant capacities is similar to earlier reports by Oboh and Umoru (2011) in some Nigerian fruits. The result further show that the high antioxidant level of 'zobo' correlated with its increased ability of the drink to scavenge free radical and protect the body against diseases.

Soybean milk drink did not differ significantly (p≥0.05) among its group in its antioxidant content. It has a fairly high polyphenols such as isoflavones which have been shown to be chemopreventive (Messina and Flickinger, 2002; Omoni & Aluko, 2005). Soybean was second to zobo drinks in the scavenging of the DPPH radical, ferric reducing ability and only slightly decolourises the ABTS radicals. Hydrolysis of soy protein improved antioxidant efficacy due to liberation of bound phenols, released from chelating agents such as phytic acid, and production of antioxidant peptide sequences (Wang *et al*., 2008).The released phenols might influence the scavenging ability of the drink. Sugar had no effect in the antioxidant content and capacity of the drinks.

Palm wine had the lowest phenols, flavonoids and vitamin C content which is in line with report by Ogunro and Ologunagba (2011) that palm wine consumption may deplete the body's antioxidants which in over whelming free radical attacks can render the body in a state of oxidative stress. The palm drinks had the lowest ferric reducing ability as a result of its inability to reduce iron (II) to iron (III), as result of the low levels of antioxidant. Although, palm

wine has reduced antioxidant content, it is a refreshing drink in local villages.

There was no significant difference amongst the kunu drink group. It thus shows that sugar had no significant impact in the antioxidant content of the drinks but only sweetens the beverage. Also, kunu drink had lower content of antioxidants when compared with zobo and soybean drinks because kunu made from millet (*Pennisetum americanum*) has a low (0.3-3.0%) polyphenols as reported by Chathan and Malleshu (2011). Kunu drinks had the lowest ability to mop up the DPPH radical and a fair ability in the mopping up of the ABTS and NO radicals. Its ferric reducing property was only slightly higher than those of the palm drinks.

Plantains are inexpensive sources of calories in Nigeria (Akubor *et al.,* 2003). Plantain drink show a relative low antioxidant content which was significantly higher than palm drinks. Plantain drinks also had significant higher levels of phenols and vitamin C. There was a fair correlation between the antioxidant content and antioxidant assays in the plantain drink.

CONCLUSION

Zobo drinks had the highest antioxidants and antioxidant scavenging capacities. This was boosted by the addition of ginger (*Zingziber officinale*). Palm wine had the lowest antioxidant capacity. Sugar has no effect in the kunu and soybean drinks. Plantain is an inexpensive source of calories with low antioxidant capacity.

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