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Total phenolics, vitamin C and free radical scavenging capacities of some Nigerian fruits

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Abstract
Aqueous extracts of selected fruits were assessed to ascertain if, there is a correlation between the natural antioxidants in the fruits and their antioxidant capacities using four in-vitro assay methods. Total phenol, flavonoids, vitamin C, 2,2-diphenyl-1-picrylhydrazyl [DPPH], 2,2'-azino-bis (3-ethylbenzthiazoline-6-sulphonic acid) [ABTS] , radical scavenging abilities, ferric reducing antioxidant property [FRAP], and iron II chelating ability were determined in the fruit extracts. Guava and shaddock contained the highest amount of total phenol (364.00±5.66mg/100g GAE) and flavonoids (83.64±5.15mg/100g) respectively. Watermelon and pawpaw had the lowest value for total phenol (14.1±2.83mg/100g GAE) and flavonoids (3.64±0.00mg/100g). Vitamin C content was highest in Guava (5.65±1.37mg/g), watermelon had the lowest amount (0.33±0.28mg/g). Shaddock and guava had the highest value for DPPH (63.78±5.89%) and ABTS scavenging ability (0.0042±0.00005mmol/g) respectively. Watermelon and lime had the lowest value for DPPH (11.22±0.45%) and ABTS scavenging ability (0.00079±0.000083mmol/g)respectively.FRAP was highest in Guava (20.54±0.02mg/g). Pawpaw had the highest value for iron chelation (41.35±1.36%), while watermelon and apple had the lowest value for FRAP (1.11±0.55mg/g) and iron chelation (11.54±0.00%) respectively. Total phenol, Flavonoid and vitamin C correlated positively with FRAP, ABTS and DPPH assays. Guava, shaddock and pawpaw possess significant antioxidant and radical scavenging abilities which correlated positively with the antioxidants.

Keywords: Antioxidants, Antioxidant capacity, free radicals, Nigerian fruits.

Introduction

Citrus fruits belong to the family of *rutaceace* and are one of the main fruit tree crops grown in Nigeria. Fruits are good sources of fiber, minerals and vitamins (Okwu and Emenike, 2006) .They are generally fat free with the exception of avocados, coconuts and olives. Fruits are good sources of natural antioxidants for the human diet, which provide protection against harmful free radicals. (Kaur and Kapoor,2001, Farombi and Fakoya, 2005).

Free radicals are highly reactive chemical molecules such as superoxide radical, hydroxyl radical and singlet oxygen that travel around the body and cause damage to the body cells. There is increased evidence that reactive oxygen species and their promoted oxidative damage are involved in a large number of diseases which includes cancer, atherosclerosis, heart disease, stroke, diabetes mellitus, rheumatoid arthritis, osteoporosis, ulcers, sunburn, cataracts and aging (Gülçin et al., 2003).

Antioxidants enzymes (made in the body) and antioxidant nutrients (found in foods) can scavenge and deactivate this reactive free radicals turning them to harmless particles (Chu et al., 2002). Improving body antioxidant status is a way to fight against degenerative diseases. This could be achieved by higher consumption of vegetables and fruits (Oboh and Rocha, 2007a). The positive effects attributable to antioxidants is the presence of carotenoids, flavonoids, lycopene, phenolics, vitamin C and

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ß-carotene (Amin et al, 2004;. Zhang and Hamauzu, 2004)

Fruits are available in all seasons in Nigeria, but despite their nutrient values, they are not usually consumed as a part of the diet in urban areas. Nigerians are becoming more aware of the important value of fruits and vegetables in their diet. Falade et al (2003), reported the organic acid and mineral available in some Nigerian fruits. Adeboye et al, (2007), reported antioxidant and antioxidant capacity in the fruit pulp of snake tomato (*Trichosanthes cucumerina L).* Atawodi *et al* (2009), Oviasogie *et al*, (2009) evaluated the polyphenol composition and antioxidant potential of *Hibiscus esculentus L* fruit and some fruits cultivated in Nigeria.

Boyer and Liu, (2002), reported a correlation between apple consumption and reduction in the risk of stroke, heart disease and lung cancer. The effectiveness of the antioxidants usually increases with their concentration (Decker *et al*, 2005).

This study aims to determine the amount of antioxidants (phenol, flavonoid and vitamin C) and investigate the comparative free radical scavenging capacity in some Nigerian fruits

MATERIALS AND METHODS

Materials

The Fruits namely; Apple (Green) (*Malus dometica*), Garden egg (*Solanum melogena*), Grape (*Citrus paradisi*), Guava (*Psidium guajava*), Lime *(Citrus aurantifolia)*, Orange *(Citrus sinensis),* Pawpaw (*Carica papaya*), Pineapple (*Ananas comosus)*, Shaddock *(Citrus maxima),* Tangerine (*Citrus reticulata),* Watermelon (*Citrullus lanatus*) were obtained from the Oba market, Benin City, Edo state, Nigeria.

Preparation of samples and extraction procedures

The fruit were prepared as normally eaten by the general populace. They were washed, peeled and cut to remove the seeds when necessary.

50 g of each prepared fruit was homogenized in an electric blender and with 50 ml of distilled water to get a uniform consistency. The slurry was poured into a measuring cylinder and the volume was made up to 100ml using distilled water. Therefore the solution was 50g/100ml (0.5g/ml). The solution was centrifuged using low speed centrifuge KX2400C for 10mins at 500rpm. The supernatant was collected and stored in 10ml plastic bottles. All the supernatant was stored in a refrigerator at 0^{o}C until utilized.

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-diphenyl–2 picrylhydrazyl) free radical was evaluated as described by Gyamfi *et al.* (1999). The extracts (300*u*l) 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 was taken at 516 nm in the JENWAY UV-Visible spectrophotometer. 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 radical 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 extract (100*u*l) was added to 2.0ml ABTS solution and the absorbances were measured at 734nm after 15mins in the JENWAY UV-Visible spectrophotometer. 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). The extract (100*u*l)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^{o}C for 20 min. and then 2.5 ml 10 % trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. 5 ml of the supernatant was mixed with an equal volume of water and 1 ml 0.1% ferric chloride. The absorbance was measured at 700 nm in the JENWAY UV-Visible spectrophotometer. The ferric reducing antioxidant property was subsequently 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 the extracts (100*u*l). 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 510nm in the JENWAY UV-Visible spectrophotometer. The Fe (II) chelating ability was subsequently calculated.

E. Determination of total phenol content

The total phenol content was determined according to the method of Singleton *et al.* (1999).The aqueous extracts(100*u*l) were oxidized with 2.5ml 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0ml of 7.5% sodium carbonate. The reaction mixture was incubated for 40minute at 45^{o}C and the absorbance was measured at 765nm in the JENWAY UV-Visible spectrophotometer. 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 reported by Meda *et al,* (2005). The aqueous extract (100*u*l) 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 subsequently measured at 415 nm in the JENWAY UV-Visible spectrophotometer; The total flavonoid content was subsequently calculated using quercetin (10mg/100ml) as standard the total

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 μl was added to 150 μl of the extracts and then 100 μl 13.3 % trichloroacetic acid (TCA) and water was added. The reaction mixture was subsequently incubated for 3 h at 37 ^{o}C, then 0.5 ml of 65 % H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm in the JENWAY UV-Visible spectrophotometer. The vitamin C content of the extracts was subsequently calculated using ascorbic acid as standard (10mg/100ml) as standard

Statistical analysis

All experiments were carried out in triplicates. Results are expressed as mean ± standard error of the means. Pearson's 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 $P \leq 0.05$.

RESULTS

The vitamin C, total phenols and flavonoid content of the fruit extracts are represented in Table 1. Guava (*Psidium guajava*) had the highest amount of vitamin C with a value of 15.65±1.37 mg/g, followed by shaddock and pawpaw with values of 14.62±0.09 mg/g and 9.81±0.64 mg/g respectively. Watermelon had the lowest amount of vitamin C with a value of 0.33±0.28mg/g. However, tangerine, apple, orange and pineapple were not significantly different at $p \leq 0.05$ in vitamin C; but pawpaw, and garden egg were significantly different at $p \leq 0.05$.

The phenolic content was highest in Guava (*Psidium guajava*) with a value of 365±5.66mg/100g gallic acid equivalent. This was followed by shaddock and orange. The lowest phenolic content was found in watermelon (*Citrullus lanatus*) with a value of 14.1±2.83mg/100g GAE. The result revealed that tangerine, apple, grape, and pineapple were not significantly different at $p \leq 0.05$.

The total flavonoid content was highest in shaddock (*Citrus maxima*) with a value of 83.65±5.15mg/100g. The lowest flavonoid content was found in pawpaw (*Carica papaya*) with a value of 3.64±0.00mg/100g. The result revealed that tangerine, apple, orange and watermelon were not significantly different at $p \leq 0.05$.

Table 1: Vitamin C, Total phenol and flavonoid content of the fruit samples

Fruit samples	VitaminC (mg/g)	TotalPhenol (mg/100g GAE)	Total Flavonoid (mg/100g)
Tangerine (*Citrus reticulata*)	4.29±0.00^{bc}	52.4±0.00^{d}	5.46±2.57^{ab}
Pawpaw (*Carica papaya*)	9.81±0.64^{e}	33.55±1.91^{bc}	3.64±0.00^{a}
Apple (*Malus domestica*)	5.33±0.23^{cd}	51.7±0.99^{d}	5.09±0.00^{ab}
Guava (*Psidium guajava*)	15.65±1.37^{f}	364.0 ±5.66^{g}	58.2±0.00^{f}
Grape (*Citrus paradise*)	6.89±2.2^{d}	53.75±22.84^{d}	36.36±0.00^{e}
Lime (*Citrus aurantifolia*)	3.64±0.18^{b}	36.9±0.99^{bcd}	25.46±0.00^{d}
Orange (*Citrus sinensis*)	5.01±0.09^{cd}	76.55±1.91^{e}	14.54±0.00^{c}
Gardenegg (*Solanum melongena)*	2.54±0.46^{b}	28.9±2.83^{ab}	10.92±0.00^{bc}
Shaddock (*Citrus maxima)*	14.62±0.09^{f}	95.3±0.00^{f}	83.64±5.15^{g}
Pineapple (*Ananas comosus)*	4.29±0.00^{bc}	46.3±2.83^{cd}	25.46±5.15^{d}
Watermelon (*Citrullus lanatus)*	0.33±0.28^{a}	14.1±2.83^{a}	7.28±5.15^{ab}

Values are mean value ± standard error of the means of triplicate determination.
Mean values with different superscript in a column are significantly different at p≤ 0.05.

The antioxidant scavenging abilities of the fruits are shown in Table 2. Shaddock (*Citrus maxima*) had the highest ability to scavenge DPPH radical with a value of 63.78±5.89%, followed by guava and orange. Watermelon (*Citrullus lanatus)* had the lowest ability with a value of 11.22±0.54%. The results revealed that the DPPH scavenging ability of shaddock, pawpaw, guava, grape, and orange were not significantly different from each other at p<0.05 while tangerine, apple, lime and pineapple were significantly different at p≤0.05.

Guava (*Psidium guajava*) had the highest ability to scavenge ABTS radical with a value of 0.0042±0.00005mmol/g while, lime (*Citrus aurantifolia*) had the lowest ability with a value of 0.00079±0.000083mmol/g. The results revealed that gardenegg and watermelon were not significantly different at p≤0.05 while tangerine, pawpaw, apple, grape, orange, shaddock and pineapple were significantly different at p≤0.05.

Guava (*Psidium guajava)* had the highest reducing power with a value of 20.54±0.02mg/g while watermelon (*Citrullus lanatus*) had the lowest reducing power with a value of 1.11±0.55mg/g. Tangerine, lime, garden egg, and pineapple were not significantly different. While pawpaw, apple, grape, orange, shaddock were significantly different at p≤0.05.

Pawpaw (*Carica papaya*) had the highest chelating ability with a value of 41.35±1.36%, while apple (*Malus domestica*) had the lowest chelating ability with a value of 11.54±0.00%. The result revealed that tangerine, watermelon, guava, lime, orange, shaddock, and pineapple were not significantly different at p<0.05 while grape and garden egg were significantly different at p≤0.05.

Table 2: Free radical scavenging abilities of the fruit extracts

Fruit samples	DPPH Scavenging ability (%)	ABTS Scavenging ability (mmol/g)	Reducing property (mg/g)	Iron chelation (%)
Tangerine (*Citrus reticulata*)	24.68±1.34^{b}	0.0023±0.00028^{c}	2.08±0.18^{a}	18.27±1.36^{a}
Pawpaw (*Carica papaya*)	56.41±10.88^{f}	0.0039±0.0013^{e}	4.61±0.52^{cd}	41.35±1.36^{c}
Apple (*Malus domestica*)	41.67±0.91^{e}	0.0017±0.00025^{b}	3.29±1.41^{b}	11.54±0.00^{a}
Guava (*Psidium guajava*)	58.65±4.08^{f}	0.0042±0.00005^{f}	20.54±0.02^{e}	15.39±2.72^{a}
Grape (*Citrus paradisi*)	54.49±3.63^{f}	0.0034±0.000028^{d}	4.16±0.15^{bc}	22.12±1.36^{a}
Lime (*Citrus aurantifolia*)	29.99±1.62^{cd}	0.00079±0.000083^{a}	1.84±0.06^{a}	13.46±2.72^{a}
Orange (*Citrus sinensis*)	57.37±1.34^{f}	0.0025±0.00015^{c}	3.54±0.18^{bc}	19.24±2.00^{a}
Gardenegg (*Solanum melongena*)	18.27±4.08^{ab}	0.00088±0.0001^{a}	1.2±0.28^{a}	32.69±2.5^{bc}
Shaddock (*Citrus maxima*)	63.78±5.89^{f}	0.0036±0.000035^{d}	5.53±0.3^{d}	18.27±1.36^{a}
Pineapple (*Ananas comosus*)	37.18±3.63^{d}	0.00196±0.0^{b}	2.12±0.16^{a}	12.5±3.08^{a}
Watermelon (*Citrullus lanatus*)	11.22±0.45^{a}	0.00096±0.000054^{a}	1.11±0.55^{a}	19.23±0.00^{a}

Values are mean value ± standard error of the means of triplicate determination.
Mean values with different superscript in a column are significantly different at p≤ 0.05.

Correlation studies

The correlation between Total phenol, flavonoid, Vitamin C and antioxidant capacities (DPPH, ABTS, FRAP, Iron chelation) is represented in Table 3. A positive correlation between total phenol and total flavonoid (r (2) =0.56) was observed. The antioxidants (Phenol, flavonoids and vitamin C) had a positive correlation with the free radical scavenging ability assays.

Total phenol correlated positively with FRAP(r (2) = 0.98) and ABTS assay (r (2) =0.59). There was a fair correlation with DPPH assay (r (2) =0.47). Total flavonoid content correlated positively with FRAP (r (2) =0.55), ABTS assay (r (2) =0.53) and DPPH assay (r (20.58).

Vitamin C correlated positively with FRAP (r (2) =0.79), ABTS assay (r(2)=0.87) and DPPH assay (r(2)=0.82).There was a fair correlation with iron chelation. The result shows that the higher the antioxidants the higher the ability to scavenge free radicals and protect the body.

Table 3: Correlation of Total phenol, Total flavonoid, Vitamin C with Free radical scavenging abilities of the fruit extracts.

	Total phenol	Total flavonoid	Vitamin C	Reducing power	ABTS Scavenging Ability	DPPH Scavenging Ability	Iron chelation
Total phenol	1						
Total flavonoid	0.56220	1					
Vitamin C	0.73166	0.77231	1				
Reducing power	0.98278	0.55032	0.78819	1			
ABTS Scavenging Ability	0.58983	0.53079	0.87453	0.67952	1		
DPPH scavenging ability	0.47233	0.58427	0.82097	0.53987	0.855517	1	
Iron chelation	-0.2433	-0.28046	0.04409	-0.1278	0.229188	0.048924	1

DISCUSSION

Fruits generally are rich sources of antioxidants (Liu et al,2002).Epidemiologic studies have demonstrated an inverse association between consumption of fruits and vegetables with morbidity and mortality from degenerative diseases (Pellegrini et al., 2003). The selected fruits analyzed in this study showed that, the fruits extracts had different free radical scavenging capacities depending on the method applied. Therefore, the fruits ranked differently depending on the assay.

Phenolic antioxidants are the most abundant antioxidant in human diet (Nagai et al,2003).They are strong antioxidant capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce alpha-tocopherol radicals and inhibit oxidases (Proteggente *et al*., 2003).

The high phenolic and vitamin C content of Guava and shaddock, could be that it contains both carotenoids and polyphenols, which are the major class of antioxidant pigments giving them relatively high antioxidant value among plant foods (Jimenez-Escrig et al., 2001). Krengsak et al (2006), reported similar results in guava fruits. The association of the fruits with a wide variety of phytonutrients which include citrus flavones, anthocyanins, hydroxycinnamic acids could play a contributory role (Amic et al., 2003). Beneficial effect of fruits, vegetables and tea or red wine has sometimes been attributed to flavonoid compounds (Ross and kasum, 2002). Flavonoids have antioxidant capacity and could therefore lower cellular oxidative stress (Oboh and Rocha, 2007b). The high flavonoid content of Shaddock, one of the citrus fruits, could be due to the red or pink inner fleshy pulp. The other tropical fruit pineapple also showed a high flavonoid content. This is in agreement with the result where a good correlation was seen between total phenolic content and flavonoid content (r(2)=0.56).

Oboh and Akindahunsi, (2004), reported that vitamin C contributed to the antioxidant activities of plant foods and exhibit its antioxidant property of electron donation. Vitamin C is the primary water soluble antioxidant in the body disarming free radicals and preventing damage in the aqueous environment both inside and outside the cell (Khaw et al., 2001).Grapefruit is a good source of vitamin C, pectin fiber and Lycopene (Lee, 2000). The low vitamin C content of Watermelon, suggests that this fruit is valuable for its carotenoid content and as a thirst quencher.

There was a positive correlation between vitamin C and total phenol (r(2)=0.73) and between vitamin C and flavonoid content (r(2)=0.77). Phenols and Flavonoids also exhibit

this antioxidant properties hence the good correlation above.

DPPH is a free radical donor that accepts an electron or hydrogen ion to become a stable diamagnetic molecule (Valdez *et al*., 2000). The high DPPH scavenging ability observed in shaddock and guava could be attributed to the vitamin C content which is a good electron donor to the DPPH radical, thereby aiding to stop their damaging effect (Je et al., 2009). This is further demonstrated in the good correlation between vitamin C and DPPH scavenging ability (r (2)=0.82).

The carotenoids and vitamin A present in guava are good singlet oxygen quenchers which donates electrons (Galano et al, 2010).This could also be responsible for the high DPPH scavenging ability. Watermelon had the lowest DPPH scavenging ability. Similar results have been reported by Buratti *et al* (2001); Halvorsen et al (2002).

The free radical scavenging ability of the fruit extracts were further studied using ABTS radical. This is because it is more versatile; as both non-polar and polar samples can be assessed (Re et al., 1999). Guava, pawpaw and shaddock had high ABTS radical scavenging abilities. The good correlation between DPPH and ABTS scavenging ability (r(2)=0.86), phenol and flavonoid content (r(2)=0.58) shows that the antioxidant is correlated with the free radical scavenging abilities. The reducing power of the fruit was assessed based on their ability to reduce iron (II) to iron (III) in the ferric reducing antioxidant property (FRAP) assay. The values in the FRAP assay will express the corresponding concentration of electron donating antioxidants (Halvorsen et al., 2002). Guava, pawpaw and shaddock had good correlation between vitamin and FRAP assay (r (2) =0.79). This may be due to the fact that vitamin C is a good electron donor and so a good reducing agent. The good correlations observed between phenols, flavonoids and FRAP,ABTS and DPPH scavenging confirmed the fact that, the antioxidant activity of the phenolic compound is mainly due to their redox properties (Nijveldt *et al*., 2001).

The iron chelating ability of antioxidants prevents metal from participating in the initiation of lipid peroxidation and oxidative stress through metal catalysed reaction (Oboh and Rocha, 2007b). The ability of the extract to chelate transition metals is therefore considered to be due to an antioxidant mechanism (Dastmachi et al., 2007).The iron chelating ability of fruits extracts could be attributed to the presence of two or more of the following functional groups: -OH, -SH, -COOH, C=O, -S-, and –O- in a favorable structure-function configuration (Yuan et al., 2005; Gulcin, 2006). Pawpaw had the highest chelating ability followed by Garden egg and then grape. There was a fair correlation between iron chelating ability and vitamin C (r (2) =0.04). The correlation between the vitamin C content and iron chelating ability may confirm that vitamin C chelates heavy metals, reduces free radicals and reduces the risk of atherosclerosis, cardiovascular diseases and some cancers (Navaro et al., 2005; Temple, 2000). The results revealed that, the high free radical scavenging capacity of the fruits is probably due to the high content of phenolic compound and vitamin C in the fruit extract (Hakkinen et al., 1999)

Conclusion

The investigation revealed that; Guava had the highest free radical scavenging activity, phenolic and vitamin C content of the fruits while shaddock had the highest flavonoid and DPPH scavenging ability. Watermelon from the *Curcubitaceae* had a low free radical scavenging activity.

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