

Evaluation of antioxidant activity and vitamin C content of selected locally available fruits in Nigeria

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ABSTRACT

Background: Fruits have become popular in Nigeria as affordable sources of vitamins and minerals. Data on the nutrient content and antioxidant potential of many of the fruits is not readily available.

Objective: This study determined the antioxidant activity and Vitamin C content of the juice of four locally available fruits in Nigeria.

Methods: Fresh fruits of *Chrisophyllum albidum* (African star apple), *Dialum guineense* (African black velvet), *Anarcadium occidentale* (cashew) and *Psidium gaujava* (guava) were sourced from markets in Lagos, Nigeria. Their juice was extracted and analysed for phytochemical components and Vitamin C content using High Performance Liquid Chromatography (HPLC) method. The antioxidant activity was assessed *in vitro* using DPPH (2,2-diphenyl-1-picrylhydrazyl) method and ferric reducing power assay. Total phenolic content was also determined.

Results: The samples contained vitamin C in the range of 123-182 mg/100 g of fruit pulp. *Psidium gaujava* samples showed the highest free radical scavenging activity while *Dialum guineense* had the strongest ferric reducing power. Total phenolic compounds were highest in the juice of *Anarcadium occidentale* (566 mg GAE/100 g) and lowest in *Dialum guineense* (106 mg GAE/100 g). Tannins, reducing sugar, carbohydrates, steroids, glycosides, proteins and saponins were detected in the samples in varying degrees.

Conclusion: The juice of the fruit samples contained high amounts of vitamin C and exhibited good antioxidant activity. They can thus be used for prevention of free radical mediated diseases.

Key words: Antioxidant, Vitamin C, Fruits, Total Phenolic Compound, Proteins

Evaluation de l'activité antioxydante et de la teneur en vitamine c de certains fruits localement Disponibles au Nigeria

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RESUME

Contexte : Les fruits sont devenus populaires au Nigeria en tant que sources abordables de vitamines et de minéraux. Les données sur la teneur en éléments nutritifs et le potentiel antioxydant de nombreux fruits ne sont pas facilement disponibles.

Objectif : Cette étude a déterminé l'activité antioxydante et la teneur en vitamine C du jus de quatre fruits disponibles localement au Nigeria.

Méthodes : Les fruits frais de *Chrisophyllum albidum* (pomme étoilée africaine), *Dialum guineense* (velours noir africain), *Anarcadium occidentale* (noix de cajou) et *Psidium gajava* (goyave) proviennent des marchés de Lagos, au Nigeria. Leur jus a été extrait et analysé pour les composants phytochimiques et la teneur en vitamine C à l'aide de la méthode de chromatographie en phase liquide à haute performance (CLHP). L'activité antioxydante a été évaluée *in vitro* en utilisant la méthode DPPH (2,2-diphényl-1-picrylhydrazyl) et l'analyse de la puissance réductrice ferrique. La teneur totale en composés phénoliques a également été déterminée.

Résultats : Les échantillons contenaient de la vitamine C dans une gamme de 123-182 mg/100 g de pulpe de fruits. Les échantillons de *Psidium gajava* présentaient la plus forte activité de piégeage des radicaux libres, alors que *Dialum guineense* avait le pouvoir réducteur le plus fort. Les composés phénoliques totaux étaient les plus élevés dans le jus de *Anarcadium occidentale* (566 mg GAE/100 g) et les plus faibles dans *Dialum guineense* (106 mg GAE/100 g). Des tanins, du sucre réducteur, des hydrates de carbone, des stéroïdes, des glycosides, des protéines et des saponines ont été détectés dans les échantillons à des degrés divers.

Conclusion : Le jus des échantillons de fruits contenait de grandes quantités de vitamine C et présentait une bonne activité antioxydante. Ils peuvent ainsi être utilisés pour la prévention des maladies à médiation par les radicaux libres.

Mots-clés : antioxydant, vitamine C, fruits, composé phénolique total, protéines

INTRODUCTION

Antioxidants are molecules that protect the human body against damage by free radicals. Free radicals are highly reactive and unstable species, produced in the body during metabolism and capable of causing damage to cells and biological systems. The human body produces antioxidants to neutralize these free radicals and counter their damaging effects on the structure and function of cells but an imbalance between free radical production and antioxidant mechanisms leads to oxidative stress. Excessive production of free radicals may be due to exposure to pollutants, alcohol, cigarette smoke, infection, medication, poor diet, radiation and environmental stress.¹ Damage caused by free radicals contributes to premature aging and leads to a host of illnesses including cancer and heart diseases among others.^{2, 3} Antioxidants being first line defence against free radical damage are therefore critical for maintaining optimum health and wellbeing.

Fruits and vegetables have become popular as an important portion of a healthy diet due to recognition of their nutritional and therapeutic value. In addition to essential macro nutrients, most fruits contain vitamins, minerals, amino acids and phenolic compounds, some of which have antioxidant activity.^{4, 5, 6} They are low in calories and fats but contain dietary fibre which helps to prevent constipation and keep the intestines healthy. Some also have high content of pectin which has been shown to have protective effect against cancers of the gastrointestinal tract.^{7, 8, 9} Fruits therefore serve as an inexpensive source of natural antioxidants and mineral supplements having valuable nutrient contents that vary according to fruit variety.

Chrysophyllum albidum is commonly called African star apple or African cherry and belongs to the family Sapotaceae. Some local names include "agbalumo" (Yoruba), "udala" (Igbo), "oche" (Edo) and "udari" (Efik). The fruit is seasonal, usually available from January through March, mainly in the Southern part of Nigeria where it is popular as a snack. The pink-coloured fleshy pulp of the ripe fruit and whitish cover of the brown seeds are the parts commonly consumed.^{10,11}

Dialium guineense (African black velvet or Black tamarind) is a wild fruit commonly consumed by rural dwellers in Nigeria as a snack. It belongs to the family Fabaceae. Locally known as "awin" in Yoruba, "icheku" in Igbo, "tsamiyar kurm" in Hausa and "uge" in Edo, it is usually available in dry season when other fruits are scarce. The fruit is small with a black, brittle, inedible

shell enclosing the orange coloured edible pulp and a hard seed (which resembles a watermelon seed) in the middle. The pulp has a sweet-sour taste and is said to be a good source of vitamin C and other micronutrients.

Anacardium occidentale L. (commonly known as cashew) is a juicy fruit belonging to the family Anacardiaceae. The oval or pear-shaped juicy fruit carries a nut encased in a kidney-shaped black/grey shell. Cashew apple as it is often called, is usually eaten fresh or the sweet but astringent juice processed into fruit drinks. The juice is said to possess various micronutrients and antioxidant activity. Roasted and salted cashew nuts are consumed as snacks.¹²

Psidium guajava, commonly known as guava, belongs to the family Myrtaceae. It is a tropical and subtropical fruit known to contain macro and micro nutrients including protein, fibre, vitamins and minerals. The oval-shaped fruit is greenish yellow in colour with numerous yellow seeds in the non-fleshy pulp.

Vitamin C is a water-soluble vitamin present in most fruits. It is essential for the formation of collagen, an important component of skin protein, tendons, ligaments and blood vessels. Vitamin C is also essential for normal wound healing, and for the repair and maintenance of cartilage, bones and teeth.^{13, 14} Its deficiency causes a disease condition known as scurvy, with symptoms such as fatigue, malaise, swollen/bleeding gums, dry splitting hair, rough dry scaly skin, reduced immunity and poor wound healing. The increased demand for healthy nutrition has led to increased quest for knowledge of chemical constituents of fruits and other foods. This study was undertaken to evaluate the Vitamin C content of the juice of four selected locally available fruits, investigate the *in vitro* antioxidant activity of the juice and screen the juice for major secondary metabolites.

METHODS

Collection and preparation of samples

Fresh fruits of *Chrysophyllum albidum*, *Dialium guineense*, *Anacardium occidentale* and *Psidium guajava* were sourced from local markets in Lagos, Nigeria.

Ripe, whole fruits of *Anacardium occidentale*, *Chrysophyllum albidum* and *Psidium guajava* were selected, washed and weighed. The peels and seeds of the *Chrysophyllum albidum* were removed, after which 300 mg of the pulp was blended in domestic blender

with distilled water. The *Psidium guajava* fruits were chopped into pieces and 300 mg of the pieces was blended with distilled water. The shell of *Dialium guineense* and the seeds were removed and 300 mg of the pulp was blended with distilled water. The juice of 15 *Anarcadium occidentale* apples was squeezed out and the volume measured. The extract from each fruit was passed through a fine sieve and volume made up to 1500 mL with distilled water. The pH determination and phytochemical analysis of each sample juice were carried out on the fresh juice before the remaining portion was stored in a freezer at -20° C for other analyses.

pH Measurement

The pH of each sample was determined using a digital pH meter (Mettler Toledo FE20).

Phytochemical analysis

The phytochemical contents of the samples were assessed using standard methods.¹⁵ All samples were tested for presence of tannins, reducing sugars, carbohydrates, terpenoids, steroids, glycosides, proteins, alkaloids, flavonoids and saponins. The presence of each component was classified as scanty, moderate or abundant depending on intensity of colour or amount of precipitate or foam.

Titrateable acidity

This was determined by acid-base titration using sodium hydroxide. Distilled water (50 mL) was added to 10 mL of each sample, 3 drops of phenolphthalein were added and the solution titrated with 0.1M NaOH to a stable pink colour. The titrateable acidity was calculated as equivalent of citric acid/100 mL of sample.

Determination of total sugar

This was measured by means of a UV/Visible spectrophotometer using the method of Chow and Landhausser (2004),¹⁶ with slight modification. Different concentrations (50, 75, 100, 125, 150, 175 and 200 µg/mL) of glucose standard in distilled water were prepared from a stock solution of 1 mg/mL. The samples were diluted 1:200 for *Chrisophyllum albidum* and *Psidium guajava* samples, and 1:400 for *Dialium guineense* and *Anarcadium occidentale* samples. To each 1 mL aliquot of dilute juice or standard glucose was added 3 mL conc. sulphuric acid. The reaction was allowed for one minute and then cooled to room temperature on a water bath. One millilitre of 2% phenol solution was added to each mixture and allowed to stand for 30 min. before centrifuging at 3000 rpm for

10 min. A control was prepared by replacing sample with distilled water. Absorbances were measured at 480 nm using distilled water as blank. The amount of sugar in the sample was calculated from the standard glucose curve and expressed in g/100g of sample.

Vitamin C Assay

The Vitamin C content of the samples was determined using HPLC method of Shafqat et al (2012)¹⁷ with some modifications, under the following conditions: Mobile phase – methanol/water, 80:20; Column – C18 (Zorbax Eclipse, 4.6 x 250 mm); Wavelength – 267 nm; Temperature – Ambient; Injection volume – 10 µl; Flow rate – 1 mL/min.

A standard calibration curve was prepared using different concentrations of standard Vitamin C (20-200 µg/mL). The juice of each sample was diluted 1:10 using the mobile phase. It was then centrifuged at 5000 rpm for 5 minutes and the supernatant was passed through a 0.43 µm syringe filter before injecting into the HPLC for analysis. The concentration of Vitamin C in each sample was calculated from the standard calibration curve and expressed in mg/100g of fruit pulp.

Antioxidant assay

Antioxidant activity of each sample was measured using the methods of Socorro et al (2010)³ with some modifications for free radical scavenging activity and ferric reducing power.

Free Radical Scavenging Activity (DPPH Method)

Different concentrations (25, 50, 100, 150 and 200 µg/mL) of Vitamin C standard were prepared in distilled water. Each concentration (2 mL) was added to 2 mL of 0.2 mM DPPH (2,2-diphenyl-1-picrylhydrazyl) solution in ethanol. The mixture was incubated at room temperature for 30 min., protected from light and the absorbance was measured at 517 nm on a UV/Vis spectrophotometer. The same procedure was followed using 2 mL of each sample. The control was 2 mL of ethanol and 2 mL of DPPH solution while ethanol was used as blank. The percent inhibition or scavenging activity was calculated using the following formula:

$$\% \text{ Inhibition} = \frac{AC - AC_s}{AC} \times 100$$

Where Ac = Absorbance of control and As = Absorbance of sample.

Ferric Reducing Antioxidant Power (FRAP)

In this method, each sample was centrifuged at 3000 rpm for 10 min. and diluted 1:10. To 2 mL of each dilute sample was added 2.5 mL of 0.2 M phosphate buffer

(pH 6.6) and 2.5 mL of K_3FeCN_6 (1%). After the mixture was incubated for 20 min. at 50° C, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 3000 rpm for 10 min. To 2.5 mL of the supernatant was added 2.5 mL of distilled water and 0.5 mL $FeCl_3$ (1%) and the sample was again centrifuged at 3000 rpm for 10 min. The absorbance was measured at 700 nm using a UV/Vis spectrophotometer. Different concentrations (5, 10, 20, 50, 100, 200, 400 and 500 mM/L) of $FeSO_4$ were prepared in distilled water and their absorbances read at 700 nm wavelength on a UV/Vis spectrophotometer. For control, the sample was replaced with buffer and distilled water was used as blank.

The reducing power of each sample was determined by comparing its absorbance with the absorbance of standard ferrous sulphate.

Total phenolic compounds

Total phenolic compounds in the samples were determined by the method of Juan et al (2013).¹⁸ Different concentrations (20, 40, 50, 60, 70 and 80 μ g/mL) of gallic acid standard were prepared in distilled water. Samples were centrifuged at 3000 rpm for 10

min. and then diluted 1:10. Sample or standard (0.5 mL) was added to 1.5 mL of Folin Ciocalteu reagent (diluted 1:10), 2 mL of 20% Na_2CO_3 was added and the mixture was incubated in the dark for 2 hr. The absorbance of each standard and sample solution was measured at 765 nm using a UV/Vis spectrophotometer. A control solution was prepared by replacing sample with distilled water. The total phenolic compounds were expressed as mg gallic acid equivalent/100g of sample.

Statistical analysis

Results were expressed as mean \pm SD. Independent t-test was used to assess statistical difference among the various fruits with confidence level at $P = 0.05$.

RESULTS

Physicochemical analysis of the samples

The results of physicochemical analysis of the samples are presented in Table 1. All samples were found to be acidic as shown in their pH and titratable acidity. Total sugar content was highest in *Chrisophyllum albidum* and lowest in *Psidium gaujava* juice. Figure 2 presents a comparison of total sugar content with acidity of the samples.

Table 1: Physicochemical parameters of the fruit juice samples

Sample Name	Mean Weight of pH Whole Fruit (g)		*Titratable Acidity (g C AE/100 mL Extract)	*Total Sugar (g/100g of Sample)
<i>Chrisophyllum albidum</i>	50.0	3.30	0.662 \pm 0.0075	13.452 \pm 0.0194 ^a
<i>Dialum guineense</i>	---	3.54	0.495 \pm 0.0079	8.378 \pm 0.0133 ^b
<i>Anarcadium occidentale</i>	79.5	4.78	0.168 \pm 0.0081	7.736 \pm 0.0186
<i>Psidium gaujava</i>	165.0	3.34	0.114 \pm 0.0150	1.264 \pm 0.0136

*Values are presented as mean \pm standard deviation; $n = 5$

^asignificantly different from all other samples at $P < 0.001$; ^bsignificantly different from Cashew at $P < 0.01$
CAE = Citric acid equivalent.

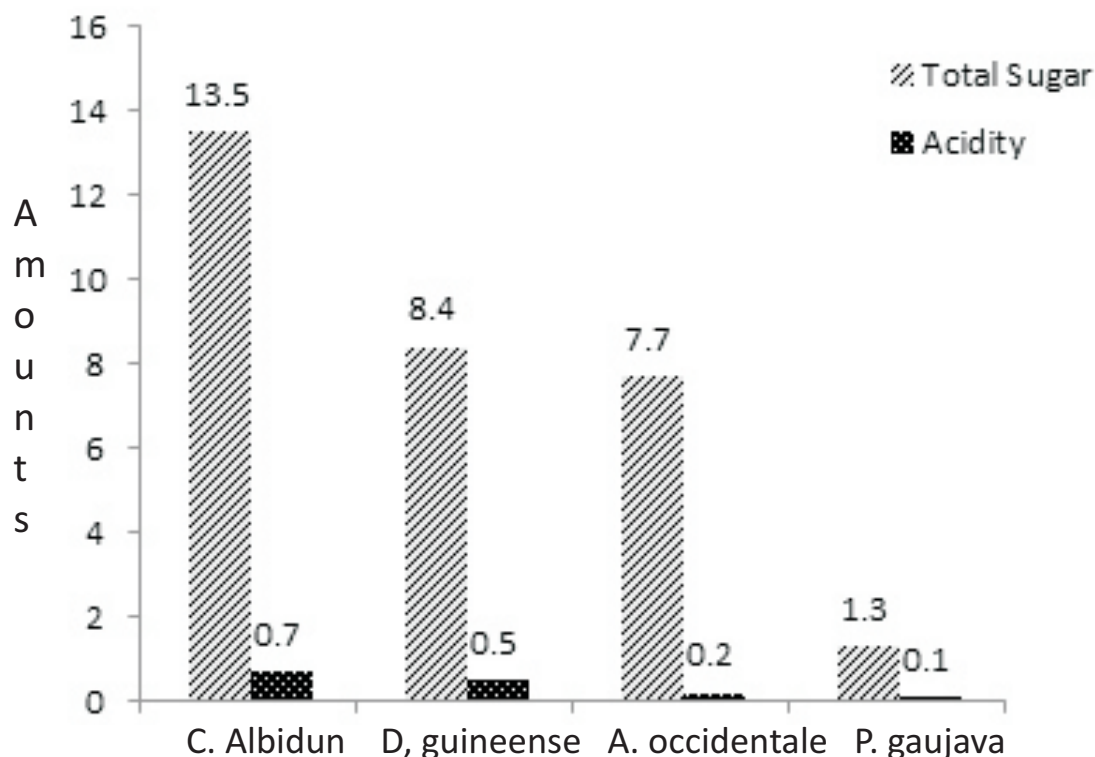


Figure 1: Comparison of total sugar content and acidity of the fruit juice samples

Phytochemical screening of samples

The results of phytochemical screening for secondary metabolites in the samples are presented in Table 2. There was abundant presence of reducing sugars, carbohydrates, steroids and glycosides in *Chrisophyllum albidum* and *Dialum guineense* while tannins, proteins and saponins were more abundant in the *Anarcadium occidentale* and *Psidium gaujava* samples.

Table 2: Results of phytochemical analysis of the fruit juice samples

Test	Chrisophyllum albidum	Dialum guineense	Anarcadium occidentale	Psidium gaujava
Tannins	-	-	+++	+++
Reducing Sugar	+++	+++	+	++
Carbohydrates	+++	+++	+	+
Terpenoids	+	-	-	-
Steroids	+++	+++	++	+
Glycosides	+++	+++	+++	+
Proteins	++	-	+++	+++
Alkaloids	-	-	-	-
Flavonoids	+	+	+	-
Saponins	-	-	++	+++

+ Scanty presence; ++ Moderate presence; +++ abundant presence

Vitamin C Content and Antioxidant Activity of Samples
 The calibration curve for Vitamin C gave a good linear curve (gradient line equation: $y = 33.695x - 239.53$ and regression (R^2) = 0.9971). The comparison between Vitamin C and total sugar content of the samples is presented in Figure 2 while the relative antioxidant activities of the samples as assessed by DPPH and FRAP methods are shown in Figure 3. The phenolic content

was compared with the free radical scavenging activity of the samples in Figure 4. *Chrisophyllum albidum* had the highest concentration of Vitamin C, *Psidium gaujava* was the best free radical scavenger while *Dialum guineense* was the most powerful ferric reducing agent. Total phenolic compounds were highest in the juice of *Anarcadium occidentale* fruit (Table 3).

Table 3: Vitamin C content and antioxidant activity of the fruit juice samples

Parameters Analysed	<i>Chrisophyllum albidum</i>	<i>Dialum guineense</i>	<i>Anarcadium occidentale</i>	<i>Psidium gaujava</i>
Vitamin C Content* (mg/100g of Fruit Pulp)	182.08 ± 0.0782	123.13 ± 0.0346	135.33 ± 0.0214	146.34 ± 0.0427
DPPH Assay (AAE in µg/mL) ^a	48.82 ± 0.0232	48.95 ± 0.0206	84.29 ± 0.0301	130.91 ± 0.0372
Ferric Reducing Power ^a (FeSO ₄ Equivalent in Mol/L)	0.834 ± 0.0206	8.72 ± 0.1364	4.37 ± 0.0214	0.592 ± 0.0204
Total Phenolic Content* (mg GAE/100 g of Fruit Pulp)	234.91 ± 0.0179	105.55 ± 0.0372	566.36 ± 0.0349	162.84 ± 0.0978

Values are mean ± standard deviation; n = 5

*Significant difference among all samples at $P < 0.001$;

^aSignificant difference among all samples at $P < 0.01$

AAE = Ascorbic acid equivalent; GAE = Gallic acid equivalent.

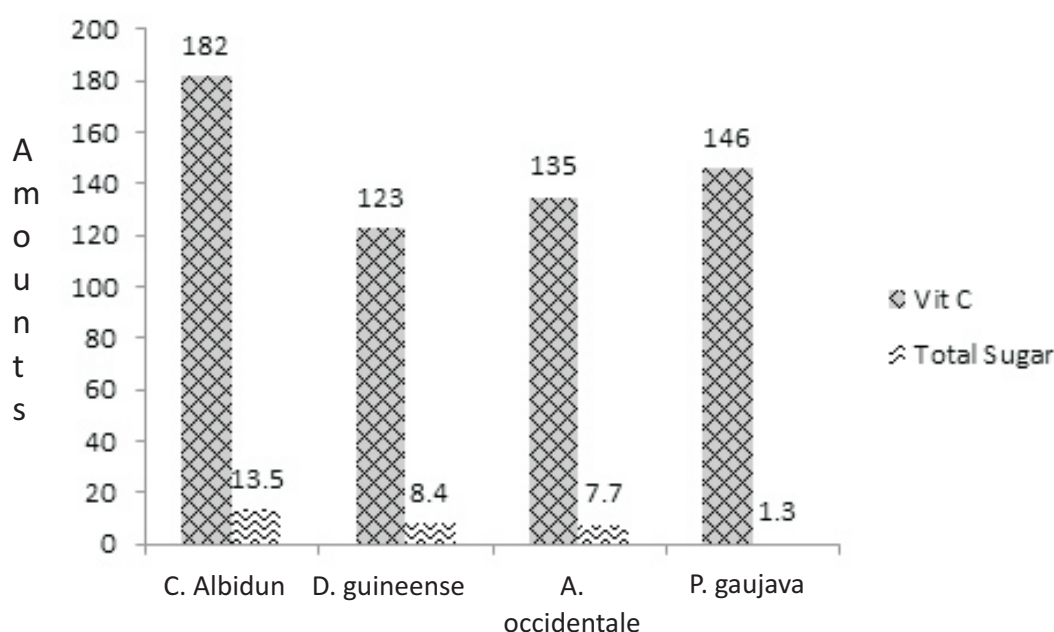


Figure 2: Relative Vitamin C and total sugar content of the fruit juice samples.

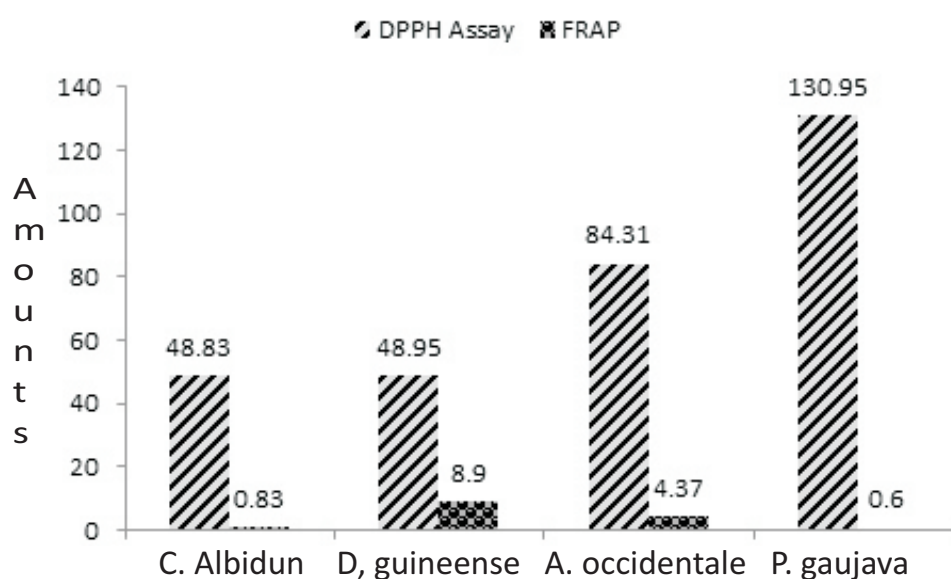


Figure 3: Relative antioxidant activity of the samples (As assessed by DPPH and FRAP methods).

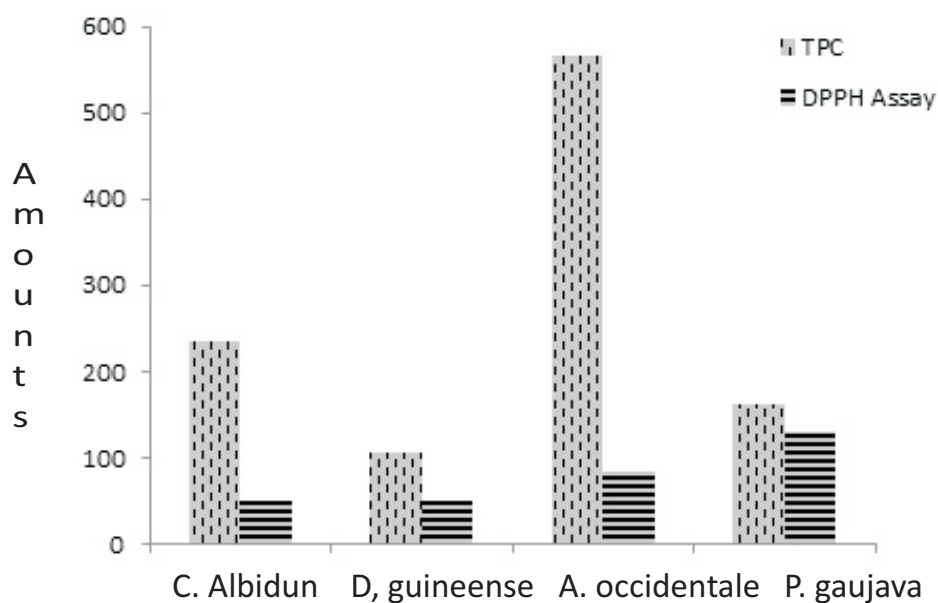


Figure 4: Total phenolic contents compared to free radical scavenging activity (Assessed by DPPH method) of the fruit juice samples. TPC = Total phenolic content.

DISCUSSION

All the samples studied were acidic with a pH range of 3.30 to 4.78 as shown in Table 1. *Anarcadium occidentale* juice was the least acidic while *Chrisophyllum albidum* was the most acidic. Values for titratable acidity also confirmed the acidity of the fruits. Acid content of fruits gives them a sour, sharp taste which is a prominent feature of the fruits studied.

Total sugar content measured in g/100 g of sample was

shown to be highest in *C. albidum* fruit juice and lowest in *Psidium gaujava* juice. There was significant difference in sugar content among the samples at $P < 0.05$. The sugar/acid ratio of fruits increases with maturity and ripening.³

The juice of *C. albidum* and *Dialum guineense* contained high amounts of reducing sugars, carbohydrates, steroids and glycosides which were found to be moderate/low in *A. occidentale* and *P. gaujava* juice. On

the other hand, tannins and saponins were high in *A. occidentale* and *P. guajava* juice but absent in *C. albidum* and *D. guineense*. Presence of proteins was high in *A. occidentale* and *P. guajava*, moderate in *C. albidum* but absent in *D. guineense*. Alkaloids were not detected in any of the samples. Some phytochemicals have been reported to possess antioxidant activity.^{19,20} Chemical composition of fruits is influenced by a number of factors such as fruit variety, geographical location, climatic conditions, soil and degree of maturity.¹⁹

The Vitamin C content was found to be highest in *C. albidum* (182 mg/100 g) and lowest in *D. guineense* (123 mg/100 g). There was significant difference in the Vitamin C content of the various fruits. A range of 125-250 mg/100g of fruit has been reported for *A. occidentale* fruit juice,²⁰ this is in agreement with current result of 135 mg/100g of fruit. Eating these Vitamin C rich fruits can therefore reduce the risk and prevalence of Vitamin C deficiency.²¹

Free radical scavenging activity, which is predictive of antioxidant activity, was determined using the deeply coloured DPPH (2,2-diphenyl-1-picrylhydrazyl) radical. *P. guajava* juice showed the highest free radical scavenging activity equivalent to 131 µg/mL of Vitamin C. *Anarcadium occidentale* juice was of moderate activity while others were of relatively low activity. DPPH is a stable free radical with a characteristic UV absorption at wavelength 517 nm.²² On reaction with an antioxidant, it is decolorized from deep purple to yellow with a loss of UV absorbance which is measured spectrophotometrically at 517 nm. The loss of absorbance was calculated as % inhibition as evidence that the DPPH radical was being scavenged by the sample. The Vitamin C standard inhibited the UV absorbance of DPPH in a dose-dependent manner showing that free radical scavenging activity increases with dose. The free radical scavenging activity of each sample was calculated as ascorbic acid (Vitamin C) equivalent in µg/mL. Therefore, the higher the value, the more potent is the sample as a free radical scavenger. The free radical scavenging activity of each fruit juice was significantly different from the others. Antioxidant activity was also assessed by measuring the ferric reducing power of the samples. The capacity

of each sample to reduce ferric to ferrous ion was determined by comparing the absorbance of the product of reduction reaction with the absorbance of standard ferrous sulphate. Therefore, the higher the value, the stronger the reducing power of the sample. The results showed that ferric reducing power was highest (8.9 Mol/L) in *D. guineense* and lowest (0.6 Mol/L) in *P. guajava* juice and the difference among the samples was significant.

The total phenolic contents in the samples were high, ranging from 106 mg GAE/100g in *D. guineense* to 566 mg GAE/100g in *A. occidentale* juice. The difference in phenolic content of the samples was statistically significant. Antioxidant activity is greatly influenced by the presence and type of phenolic compounds which are mainly phenolic acids, phenolic diterpenes and flavonoids.^{18, 23, 24} The test for phenolic compounds was based on their reaction with Folin-Ciocalteu reagent using gallic acid as standard.

The number and variety of fruits selected for this research was limited to those in season during the period of the study.

CONCLUSION

The results of this study show that the juices of the four selected fruits (*Chrisophyllum albidum*, *Dialum guineense*, *Anarcadium occidentale* and *Psidium guajava*) possess *in vitro* antioxidant activity expressed by their free radical scavenging activity and ferric reducing power. Total phenolic contents of the samples were high (106-566 mg GAE/100g of fruit pulp). The samples also contain Vitamin C in the range of 123 to 182 mg/100 g sample. It may be concluded that these fruits have good nutritive value and are recommended for human consumption as part of a balanced diet for prevention of diseases, especially those related to oxidative stress. With further studies on preservation and storage, the fruits can be processed into food supplements for health maintenance.

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