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COMPARATIVE *IN VITRO* ANTIOXIDANT PROPERTIES OF WATER JUICE FROM SELECTED AFRICAN FRUITS CONSUMED IN CALABAR, CROSS RIVER STATE (CRS), NIGERIA

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ABSTRACT

This study is based on a comprehensive comparison among different African fruits relative to one another and to identify the ones with high antioxidant capacity as compared to the standard ascorbic acid. The antioxidant capacity was analyzed using ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl(DPPH) radical spectrophotometric assays. The results of the DPPH assay indicated the antioxidant capacity as follows (decreasing order): Soursop (*Annona muricata*) (81.81% at 400ug/ml), sweet orange (*Citrus sinensis*) (75.62% at 400ug/ml), cocoa (*Theobroma cacao*) (72.26% at 400ug/ml), watermelon (*Citrulluslanatus*) (70.50% at 50ug/ml), chivita (70.03% at 400ug/ml), garden egg (*Solanummelongena*) (65.58% at 100ug/ml), pawpaw (*Carica papaya*) (65.53% at 100ug/ml), surinam cherry (*Eugenia uniflora*) (65.00% at 400ug/ml), tomatoes (*Solanumlycopersicum*) (64.99% at 400ug/ml), malay apple (*Syzygiummalaccense*) (61.67% at 200ug/ml), tangerine (*Citrus tangerina*) (61.42% at 400ug/ml), monkey cola (*Cola millenii K. Sckhum*) (60.88% at 100ug/ml), lime (*Citrus aurantifolia*) (52.87% at 100ug/ml), cucumber (*Cucumissativus*) (51.71% at 400ug/ml). The antioxidant capacities of the fruit juices were however, less when compared to the standard ascorbic acid. The FRAP assay result revealed that only the juices from African star apple (*Chrysophyllumafricanum*) and cocoa (*Theobroma cacao*) were significantly (p<0.05) higher than the standard ascorbic acid at 400ug/ml. The fruit juices have been demonstrated as good sources of natural antioxidants, hence can be exploited in the production of functional foods.

KEYWORDS: 2,2-diphenyl-1-picrylhydrazyl (DPPH), ferric reducing antioxidant power (FRAP), antioxidants, fruits, water juice, Calabar.

INTRODUCTION

Antioxidants are intimately involved in the prevention of cellular damage the common pathway for cancer, aging, and a variety of diseases. Antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction involving the loss of electrons or an increase in oxidation state. Oxidation reactions can produce free radicals which in turn, can start chain reactions. When the chain reaction occurs in a cell, it can cause damage or death of the cell. Antioxidants terminate these chain reactions by removing free radical intermediates, and inhibit other oxidation reactions(Saxena, 2014). They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid, or polyphenols. Oxidative stress is induced *in vivo* by increased production of reactive oxygen or nitrogen species(Wachtel-Galor, Siu, & Benzie, 2014). A growing body of scientific evidence has suggested that oxidative stress is involved in many human diseases, such as cardiovascular diseases and some types of cancers (Camarda *et al.,* 2007; Guo, *et al.,* 2003; Singh *et al.,* 2002). Dietary antioxidants are believed to be effective in protection against oxidative damage (Chinaka *et al.,* 2013). Fruits and vegetables are rich in vitamins, minerals, and fibres, as well as natural antioxidants such as carotenoids, phenolics, and flavonoids. These antioxidants are effective in scavenging various free radicals, inhibiting initiation of chained reaction, or suppressing formation of free radicals by binding to the metal ions (Giwa *et al.,* 2012; Gundgaard *et al.,*2003). Many epidemiological studies showed a significant inverse correlation between the intake of fruits and vegetables, and the incidence of some chronic diseases (Huxley & Neil, 2003). Therefore, increase in the consumption of fruits and vegetables have been frequently recommended to be

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one of the strategies in the prevention against oxidative stress related diseases (Kaliora *et al.,* 2006).

Previously, researchers measured the FRAP value of peel, pulp, and seed fractions of some fruits and found that the peel and seed fractions were stronger than the pulp fractions in antioxidant capacity, suggesting that the peel and seed fractions are potentially more valuable sources than the pulp fractions in terms of natural antioxidants(Kumar, et al., 2010). Similar results have been reported(Singh *et al.,* 2002), in which they found that the methanol extract of pomegranate peel had much higher antioxidant capacity than that of seeds as demonstrated by using the β-carotene-linoleate and DPPH model systems. Toor and Savage(2005) found that the skin fraction of tomatoes had a stronger antioxidant activity than the pulp fraction.

The fruits investigated in this study include: *Carica papaya* (Pawpaw), *Solanummelongena* (Garden egg), *Cucumissativus* (Cucumber), *Syzygiummalaccense* (Malay apple), *Citrus sinensis* (Sweet orange), *Citrulluslanatus* (Watermelon), *Theobroma cacao* (Cocoa), *Solanumlycopersicum* (Tomatoes), *Citrus aurantifolia* (Lime orange), *Citrus tangerine* (Tangerine), *Eugenia uniflora* (Surinam cherry), *Cola millenii k. Sckhum* (Monkey cola), *Persea Americana* (Avocado), *Annona muricata* (Soursop), *Citrus maxima* (Shaddock) and *Chrysophyllumafricanum* (African star apple).In this study the ferric reducing antioxidant power(FRAP) assay and 1,1-diphenyl-2-picrylhydrazyl(DPPH) radical spectrophotometric assay were used to determine the antioxidant capacity of different African fruits. The correlations between the different fruits and the standard ascorbic acid all at different concentrations were analyzed. The objective of this study is to make a comprehensive comparison of antioxidant capacity of different African fruits relative to one another including Chivita, a fruit juice marketed in Africa and compare these to the standard - ascorbic acid.

MATERIALS AND METHODS

Collection of Plant Material

A total of 17 fruits of different plant species were obtained in November, 2014 from Watt Market, Calabar South, Cross River State of Nigeria. The plantswere identified and authenticated in the Department of Botany, Faculty of Sciences, University of Calabar. The fruits were properly washed and rinsed with tap water to remove dirt and debris. The fruits were peeled and cut into bits and the seeds removed into separate containers. The pulps of the fruits were blended into different beakers using an electric fruit blender. The fruit juices were filtered each into different sets of labeled beakers using cheese cloth, and boiled briefly at 50^0C for 3minutesto avoid contamination by microbes and allowed to cool, upon cooling it was well shaken and allowed to stand and then filtrates were further filtered using filter paper (Whatman No. 4) into different containers of 5ml each. These fruit juice extracts were refrigerated at 4^0C.

1,1-Diphenyl-2-picrylhydrazyl Radical (DPPH) Spectrophotometric Assay.

The free radical scavenging activity of extracts was analyzed by the DPPH assay as described by Chinaka and colleagues, and Ohr (Chinaka *et al.,* 2013; Ohr, 2004). A given volume (2ml) of the extract at varying concentrations ranging from 10-500µg/ml each was mixed with 1ml of 0.5mM DPPH (in methanol) in a cuvette. The absorbance at 517 nm was taken after 30 minutes of incubation in the dark at room temperature. The experiment was done in triplicate. The percentage antioxidant activity was calculated as follows:

% Antioxidant Activity [AA] = 100 – [{(Abs sample – Abs blank) x 100}/Abs control].

Methanol (1.0 ml) plus 2.0 ml of the extract was used das the blank while 1.0 ml of the 0.5mM DPPH solution plus 2.0 ml of methanol was used as the negative control. Ascorbic acid was used as reference standard(Ohr, 2004).

Ferric Reducing Antioxidant Power (FRAP) Assay

The total antioxidant potential of sample was determined using a ferric reducing ability of plasma (FRAP) assay as described by Chinaka and colleagues, and Ohr(Chinaka *et al.,* 2013;Ohr, 2004)as a measure of "antioxidant power". FRAP assay measures the change in absorbance at 593nm owing to the formation of a blue colored Fe^{2+} -tripyridyltriazine compound from colorless oxidized Fe^{3+} formed by the action of electron donating antioxidants. Standard curve was prepared using different concentrations (100 *x* 1000 µmol/L) of $FeSO_4 \bullet 7H_2O$. All solutions were used on the day of preparation. In the FRAP assay, the antioxidant efficiency of the extracts under the test was calculated with reference to the reaction signal given by an Fe^{2+} solution of known concentration, this representing a one electron exchange reaction. Ascorbic acid was measured within 1 hour after preparation. The sample to be analyzed was first adequately diluted to fit within the linearity range. All determinations were performed in triplicate.

STATISTICAL ANALYSIS

All measurements were carried out in triplicate. Data were presented as *mean* ± *SD*. Data were collected and represented using different concentrations ranging from 50 – 400ug/ml, and compared alongside the standard (Ascorbic acid). The mean of the repeated data were calculated and the percentage antioxidant activity in DPPH assay derived. While the values of FRAP value were calculated using the formula as given by Chinaka and colleagues, and Ohr (Chinaka *et al.,* 2013; Ohr, 2004). Significant difference was accepted at $p<0.05$.

RESULTS

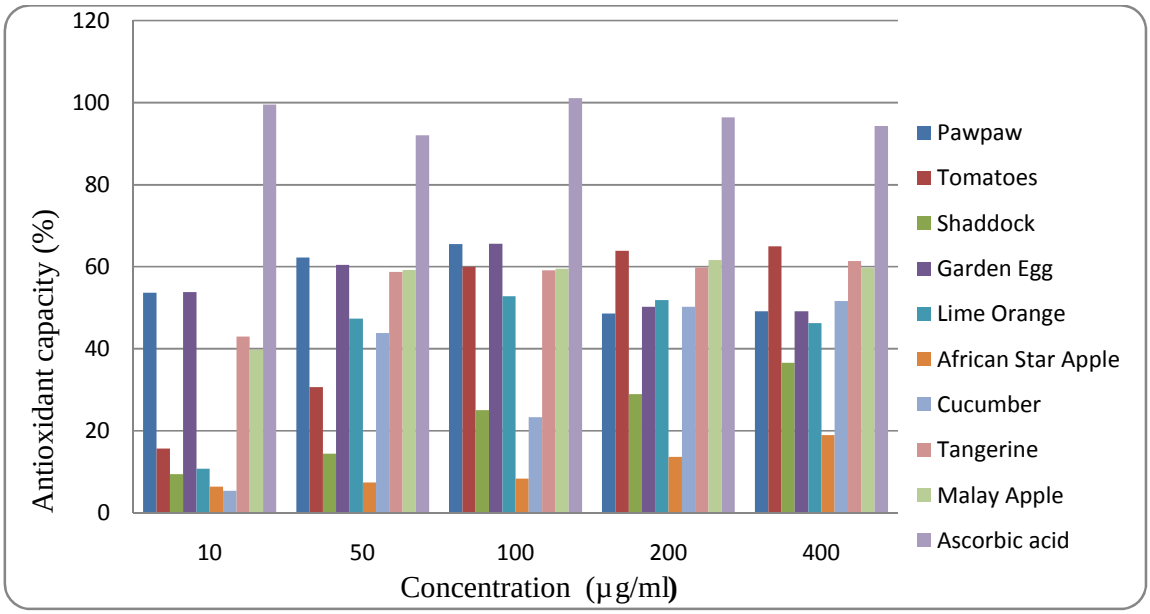

Figure 1a: Antioxidant capacities of aqueous fruit juice extracts using the DPPH assay

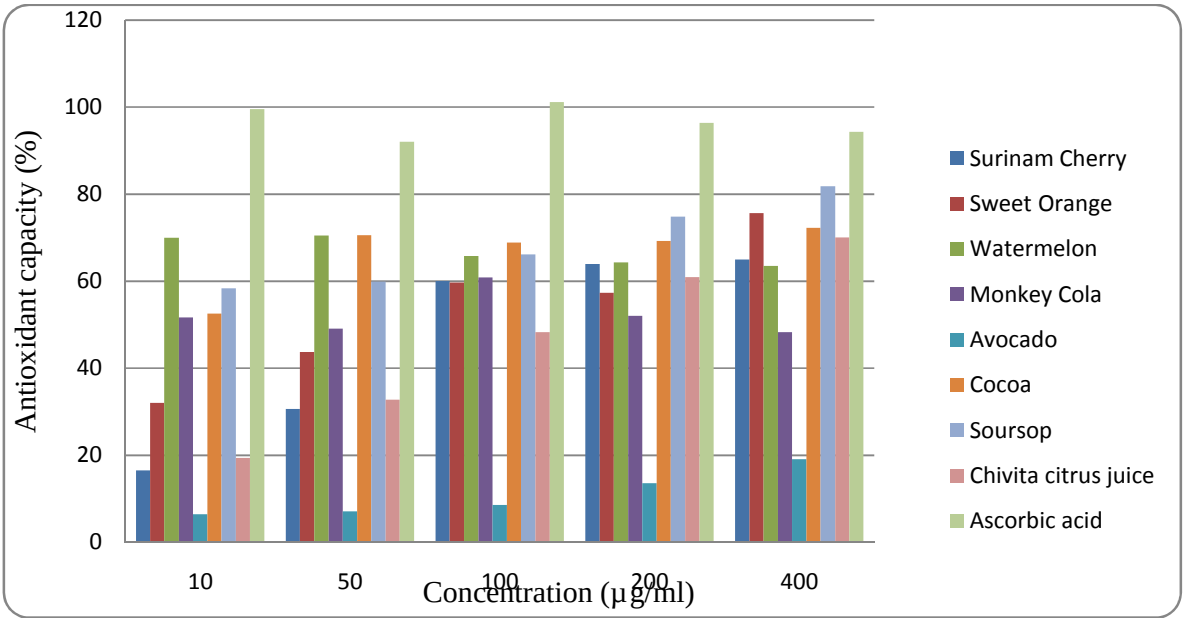

Figure 1b: Antioxidant capacities of aqueous fruit juice extracts using the DPPH assay

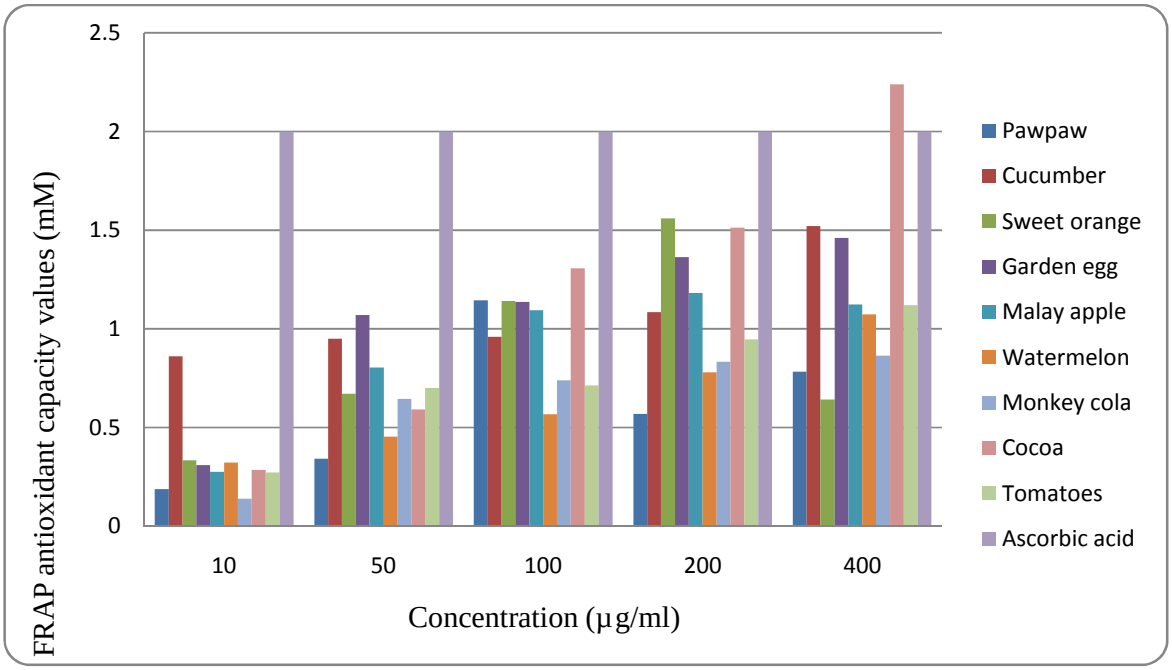

Figure 2a. Antioxidant capacities of aqueous fruit juice extract using the FRAP assay

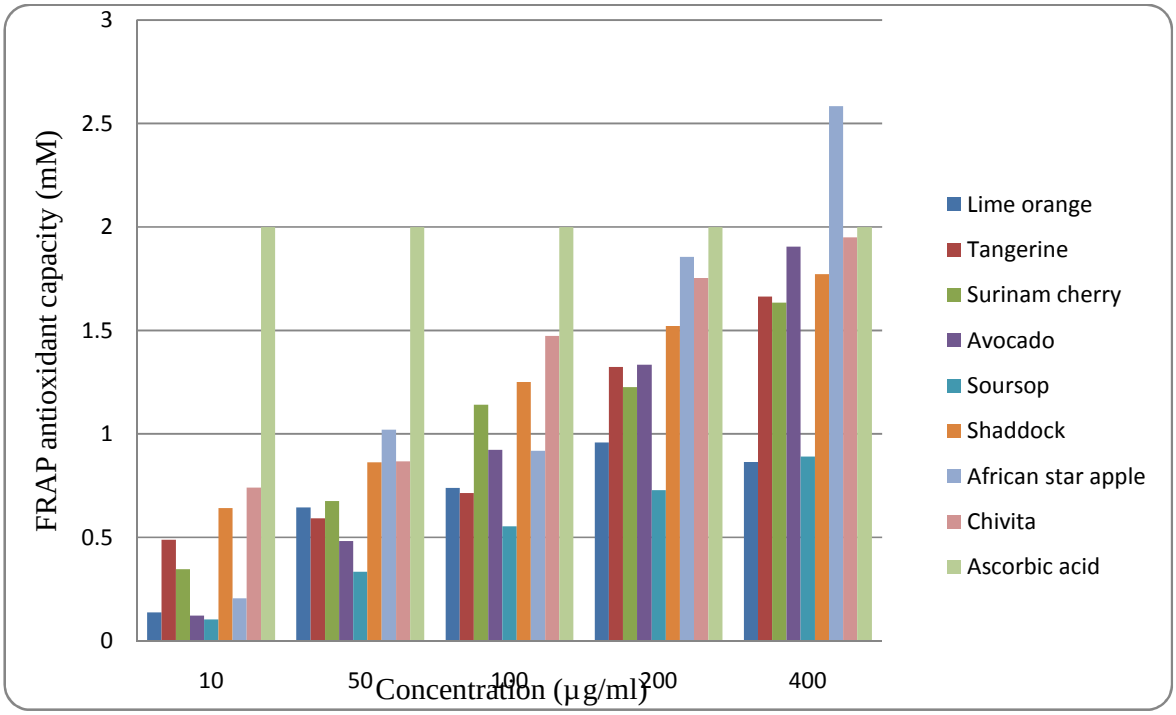

Figure 2b: Antixidant capacities of aqueous fruit juice extract using the FRAP assay

DISCUSSION

The DPPH values of 17African fruit juice extracts are shown in Fig. 1a and Fig. 1b above. The antioxidant capacity of ascorbic acid was significantly ($p<0.05$) higher at all concentrations compared to values obtained for sweet orange (*Citrus sinensis*) (75.62% at 400ug/ml), cocoa (*Theobroma cacao*) (72.26% at 400ug/ml), watermelon (*Citrulluslanatus*) (70.50% at 50ug/ml), Chivita (70.03% at 400ug/ml), garden egg (*Solanummelongena*) (65.58% at 100ug/ml), pawpaw (*Carica papaya*) (65.53% at 100ug/ml), Surinam cherry

(*Eugenia uniflora*) (65.00% at 400ug/ml), tomatoes (*Solanumlycopersicum*) (64.99% at 400ug/ml), malay apple (*Syzygiummalaccense*) (61.67% at 200ug/ml), tangerine (*Citrus tangerina*) (61.42% at 400ug/ml), monkey cola (*Cola millenii k. sckhum*) (60.88% at 100ug/ml), lime (*Citrus aurantifolia*) (52.87% at 100ug/ml), cucumber (*Cucumissativus*) (51.71% at 400ug/ml), except for Soursop (*Annona muricata*) (81.81% at 400ug/ml) at 100ug/ml. However, the juice of soursop (*Annona muricata)*ranked the highest in antioxidant capacity (81.81% at 400ug/ml) among all other fruits, while the antioxidant capacities of African star apple (*Chrisophyllumafricanum*) and avocado (*Perseaamericana*) showed significant ($p<0.05$) decrease when compared to other fruit juices extracts and ascorbic acid.

The FRAP values of 17 different African fruit juice extracts are also shown above in Fig. 2a and Fig. 2b. The results demonstrate a decrease in antioxidant capacities of the different fruit juice extracts relative to each other as arranged in descending order of magnitude: the antioxidant capacities of African star apple (2.58mM at 400ug/ml), cocoa (2.24mM at 400ug/ml), Chivita (1.95mM at 400ug/ml), Avocado (1.91mM at 400ug/ml), Shaddock (1.77mM at 400ug/ml), Tangerine (1.66mM at 400ug/ml), Surinam Cherry (1.64mM at 400ug/ml), Sweet orange (1.56mM at 200ug/ml), (1.52mM at 400ug/ml), Garden egg (1.46mM at 400ug/ml), Malay apple (1.18mM at 200ug/ml), Pawpaw (1.15mM at 100ug/ml), Tomatoes (1.12mM at 400ug/ml) and Watermelon (1.07mM at 400ug/ml). The antioxidant capacities of Lime orange (0.96mM at 400ug/ml), Soursop (0.89mM at 400ug/ml) and Monkey cola (0.86mM at 400ug/ml), showed significant ($p<0.05$) decrease when compared to other fruit juices extracts. The high vitamin C content in sweet orange (*Citrus sinensis*) is the most important medicinal active principle in the fruit. Vitamin C acts as an antioxidant in the body, which can potentially prevent conditions such as cancer, heart disease, and arthritis(Scalbert *et al.,* 2005).

Tomato contains anthocyanin, carotene and lycopene which help prevent prostate cancer (Aldrich *et al.,* 2010) and also has been shown to improve the skin's ability to protect against harmful UV rays, and that the lycopene in tomatoes might help in managing human neuro-degenerative diseases. The lycopene from tomatoes has no effect on the risk of developing diabetes, but may help relieve the oxidative stress of people who already have diabetes(Toor & Savage, 2005). The phytochemical analyses of the pulp and seed of *Cola millenii k. Sckhum*(monkey cola)showed that the extract contained alkaloids, tannins, saponins, cardiac glycosides, carbohydrate, sterol, resin and terpenes that could be responsible for the observed antimicrobial activities(Giwa *et al.,* 2012). These phytochemicals reduce the low density lipoprotein concentration (the cholesterol involved in depositing fat in the arteries), prevent blood clotting which can reduce risk of heart attack and stroke(Aldrich, et al., 2010). Kumar and friends (Kumar*et al.,* 2010)reported that the aqueous fruit extract of *Cucumissativus* L. (Cucumber) has shown strong analgesic action in mice, by inhibiting the acetic acid-induced within. Upon preliminary phytochemical screening, the aqueous extract of *Cucumissativus* fruit was found to contain glycosides, steroids, carbohydrates, saponins, and tannins. It is reported that phytochemical compounds such as flavonoids and tannins found in the cucumber (*Cucumissativus*) extract might be responsible for free radical scavenging and analgesic effects(Kris-Etherton, *et al.*, 2002). Lime fruit contains very high amounts of vitamin C and citrus bioflavonoids, vitamin C is a powerful antioxidant and natural vitamin C is much more effective than the synthetic ones because natural nutrients are more beneficial than synthetic ones. The lime fruit has been used to cure all kinds of common sickness such as catarrh, malaria, cholera, diphtheria, typhoid, cold and other deadly disease (Oriola, 2013). Drinking freshly squeezed lime in the morning helps to stimulate the liver to detoxify. However, when doing this one should not take concentrated lime, it should be diluted with water before taking it. Pure lime juice is high in acid which may be injurious to the enamel of our teeth.

CONCLUSION

It was found that the different fruit juice extracts were remarkably different in antioxidant capacity. Using the DPPH assay, Sour sop, Sweet orange, Cocoa and Watermelon, showed antioxidant activity significantly higher than that of Chivita fruit juice (citrus brand) which is one of the most popular fruit juice drinks marketed in Nigeria and can be concluded that the antioxidant capacities of fruits consumed in Nigeria such as sour sop, sweet orange, cocoa and watermelon were significantly ($p<0.05$) comparable to Chivita fruit juice than that of other fruits used. On the contrary using the FRAP assay, African star apple has the highest value ($p<0.05$) of antioxidant capacity when compared to other fruits including the standard ascorbic acid. This study provides sufficient scientific evidence that these fruits may serve as better constituents of human diet supplying the body with the required antioxidant potentials. The antioxidant potentials exhibited by the fruits under study are not unrelated to their phytochemical properties which support their traditional use in treatment of diseases such as cancer, cardiovascular disease, stroke and premature ageing by reducing the oxidative processes that leads to premature ageing. These results are also expected to guide the fruit consuming public, dietitians, medical and other health care providers on the nutritional benefits and health providing status of the fruits under study. These data can also be used to aid dietary planning in order to promote higher antioxidant intake. Further investigations and studies should be carried out on the other fractions of the fruits used in this work such as the seeds, peels, and the fibre.

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