

Research Article

Phenolic Content and *in vitro* Antioxidant Activities of Sweet Orange (*Citrus sinensis* L.) Fruit Wastes**Oikeh E.I.*¹, Oriakhi K² and Omoregie E.S¹**¹Department of Biochemistry, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.²Department of Medical Biochemistry, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria.Accepted in final form: 10th June 2014**Abstract**

The phenolic contents and *in vitro* antioxidant activities of waste components (flavedo, albedo and seed) obtained from *Citrus sinensis* (orange) fruit were evaluated in this study. Two extracts (ethanol and hydroethanol) were prepared from each sweet orange component. Total phenolic contents were higher in the ethanol seed and albedo extracts (13.05 ± 0.35 and 34.57 ± 0.09 mg gallic acid equivalent/g of extracts, respectively) compared to the hydroethanol extracts (7.88 ± 0.88 and 32.07 ± 0.67 mg gallic acid equivalent/g of extracts, respectively). The hydroethanol flavedo extract however contained significantly higher total phenolics (48.17 ± 1.17 mg gallic acid equivalent/g of extract) compared to the ethanol flavedo extract (12.50 ± 0.50 mg gallic acid equivalent/g of extract). Total flavonoid content ranged from 14.29 ± 0.38 mg quercetin equivalent/g of extract in the ethanol seed extract to 45.79 ± 1.17 mg quercetin equivalent/g of extract in the hydroethanol flavedo extract. DPPH radical scavenging activity testing revealed that the hydroethanol extracts were better inhibitors of the DPPH radical than the ethanol extracts. IC₅₀ values ranged from 0.18 - 0.23 mg/mL for the hydroethanol extracts and from 0.26 - 0.34 mg/mL for the ethanol extracts. The hydroethanol extract of the flavedo was found to have the best ferric reducing antioxidant potential (FRAP) value (800.30 ± 1.53 μ mole Fe(II)/g extract) while the seed extracts contained the lowest FRAP values (329.00 ± 1.53 and 464.30 ± 0.75 μ mole Fe(II)/g extract for ethanol and hydroethanol extracts, respectively). Reducing power was observed to be higher in the hydroethanol extracts than the ethanol extracts. Inhibition of lipid peroxidation was highest in the ethanol extract of the flavedo (29.46 ± 0.08 %) and lowest in the seed extract counterpart (8.46 ± 0.15 %). Our results suggest that wastes (flavedo, albedo and seeds) generated from sweet orange (*Citrus sinensis*) have biologically important components that may be beneficial to health.

Keywords; *Citrus sinensis*, antioxidant, DPPH, albedo, flavedo**INTRODUCTION**

Orange (*Citrus sinensis* L.), a member of the Rutaceae family, is the world's most widely grown and commercialized citrus species. Citrus fruits though classified as berries, are more often described as hesperidium. Orange fruit is mostly recognised for its vitamin C content. It is also an important source of other phytochemicals such as phenolics and carotenoids with reputed health benefits. The juice is also known to contain organic acids such as citric and malic acids (Ezejiofor *et al.*, 2011).

The orange fruit is mostly consumed for its highly nutritious juice. After juice extraction, the citrus wastes obtained accounts for about half of the total fruit. Citrus wastes consist of the outer peel (flavedo), inner peel (albedo); a white spongy layer below flavedo and the seeds.

Peel by-products have been reported to contain sugars, fiber and many other components that offer excellent opportunities as value-added products. The albedo layer is a source of pectin, which has wide applicability in the food industry as gelling in jam and jellies, thickening, texturizing, emulsifier and stabilizing agents in dairy products, fruit preparation or in icings and frostings (Hashmi *et al.*, 2012).

Flavedo and albedo wastes are a huge nuisance both industrially and domestically. In Nigeria where orange is one

of the most commonly available fruits with no organised pollution control measures, wastes from orange are discarded indiscriminately with attendant pollution problems.

Citrus sinensis peel contains calcium, phosphorus, potassium, ascorbic acid, and vitamin A (Lawal *et al.*, 2013). The peel also contains a range of essential oils which are known to have antibacterial activities. Citrus peels are also known to possess anti diabetic and anti peroxidative effects due to the high content of total polyphenols (Naim *et al.*, 2012). Industrially, citrus peel essential oils enjoy wide applicability. They are used in fragranced products such as toothpastes, mouthwash, candles and soaps. In the cosmetic industries, orange peel essential oil with characteristic fresh orange-fruity odour impressions are used in shampoos, soaps, shower gels, body lotions, deodorants and perfumes (Ezejiofor *et al.*, 2011).

Osfor *et al.* (2013) demonstrated that citrus albedo powder contains hypocholesterolemic and hypoglycaemic effects in rats. Citrus albedo may also find usability as a nutritive additive. Eldemery (2010) showed that the addition of orange albedo to beef burger improved the nutritional properties of the burger; which also enjoyed sensory and textural taste properties and over all acceptability by the tasters used in the experiment. Nassar *et al.* (2008) in their work demonstrated that highly acceptable biscuits could be obtained

by incorporating 15% orange pulp and peel in the formulation. The biscuits formulated were shown to have enhanced fibre and reduced fat content.

Citrus seeds also enjoy some usability. Sumroiphon *et al.* (2006) showed that they may be used in mosquito-borne larval control. This may be of great importance in tropical areas of the world where malaria-causing vectors thrive. It has also been demonstrated to contain significant *in vitro* and *in vivo* antimicrobial activities (Ionescu *et al.*, 1990).

Antioxidants from natural sources are preferable because they are deemed to be safe and free from toxicities associated with synthetic antioxidants. Natural antioxidants belonging to higher plants especially the typical compounds, such as vitamins, carotenoids and phenolics exhibit antioxidant activity and they reduce disease-associated chronic health problems. It has also been suggested that an inverse relationship exists between antioxidative status and incidence of human diseases such as cancer, aging, and atherosclerosis (Rahman *et al.*, 2013).

Recent interest in the investigation into medicinal plants with the aim of discovering natural antioxidants with pharmacological importance is rising. This study therefore investigates the phenolic content and *in vitro* antioxidant activity of waste portions of *Citrus sinensis* fruit as a basis for the extraction of medicinally important by-products from it..

MATERIALS AND METHODS

Raw materials

Fresh oranges were purchased from a market in Benin City, Nigeria in May 2013. The fruits were washed with distilled water and the peels removed with the aid of a sharp knife. Outer peel removal was carried out to ensure that the flavedo was not harvested alongside the albedo. The juice was extracted with the aid of a juice extractor and the seeds collected. Residual juice sacs were removed to generate the flavedo. The albedo, flavedo and seeds were air-dried in a shade at room temperature and then pulverized.

Preparation of Extracts

Extracts of flavedo, albedo and seeds were prepared using two different solvent systems; absolute ethanol and 70% ethanol: distilled water (v/v) (hydroethanol). Each extract type was prepared by soaking the citrus wastes in their respective solvents in air tight containers for 72 hours with occasional stirring. The extracts were then filtered and taken to a rotatory evaporator (RE 300, Bibby Scientific, UK) for concentration at low pressure. The extracts were thereafter stored in a refrigerator till they were required.

Determination of Total Phenolic Content

Total phenolic content was determined according to Folin and Ciocalteu reagent method of Cicco *et al.* (2009). Concentrations of 0.2, 0.4, 0.6, 0.8, and 1 mg/mL of gallic acid were prepared in methanol. Concentrations of the extracts were also prepared in methanol. Then 4.5 mL of distilled water was added to 0.5 mL of the extract and mixed with 0.5 mL of a ten-fold diluted Folin- Ciocalteu reagent. Subsequently, 5 mL of 7% sodium carbonate and 2mL of distilled water were added. The mixture was allowed to stand for 90 min at room temperature before the absorbance was read at 760 nm. All determinations were performed in triplicates with gallic acid utilized as the positive control. The total phenolic content was expressed as gallic acid equivalent (GAE).

Determination of Total Flavonoid Content

Total flavonoid content was determined using the method of Miliuskas *et al.* (2004). Briefly, 2 mL of 2% AlCl₃ in ethanol was added to 2 mL of extracts (concentrations of extracts were 0.1 – 1.0 mg/mL), in methanol. The absorbance was measured at 420 nm after one hour incubation at room temperature. Similar concentrations of quercetin, the positive control were used. The total flavonoid content was calculated as mg quercetin equivalent /g of extract.

Estimation of Diphenyl-2-picryl-hydrazyl (DPPH) Radical Scavenging Activity

The free radical scavenging capacity of the plant extracts against 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined by a slightly modified method of Brand-Williams *et al.* (1995). Briefly, 0.5 mL of 0.3 mM DPPH solution in methanol was added to 2 mL of various concentrations (0.2 - 1.0 mg/mL) of the extracts. The reaction tubes were shaken and incubated for 15 min at room temperature in the dark, and the absorbance read at 517 nm. All tests were performed in triplicate. Vitamin C was used as standard control, with similar concentrations as the test samples prepared. A blank containing 0.5 mL of 0.3 mM DPPH and 2 mL methanol was prepared and treated as the test samples.

The radical scavenging activity was calculated using the following formula:

$$\text{DPPH radical scavenging activity (\%)} = \frac{[(A_0 - A_1) / (A_0)] \times 100}{}$$

Where A₀ is the absorbance of DPPH radical + methanol; A₁ is the absorbance of DPPH radical + sample extract or standard. The 50% inhibitory concentration value (IC₅₀) was calculated as the effective concentration of the extract that is required to scavenge 50% of the DPPH free radicals.

Ferric ion Reducing Antioxidant Power (FRAP) Assay

A modified method of Benzie and Strain (1996) was adopted for the ferric reducing antioxidant power (FRAP) assay which depends on the ability of the sample to reduce the ferric tripyridyltriazine (Fe (III)-TPTZ) complex to ferrous tripyridyltriazine (Fe(II)- TPTZ) at low pH. Fe (II)-TPTZ has an intensive blue colour which can be read at 593 nm. 1.5 mL of freshly prepared FRAP solution (25 mL of 300 mM acetate buffer pH 3.6, 2.5 mL of 10mM 2,4,6-tripyridyls- triazine (TPTZ) in 40mM HCl, and 2.5 mL of 20 mM ferric chloride (FeCl₃ · 6H₂O)solution) was mixed with 1 mL of the extracts at concentrations of 0.1 - 1.0 mg/mL. The reaction mixtures were incubated at 37 °C for 30 min and increase in absorbance at 593nm measured. FeSO₄ was used for the calibration curve and ascorbic acid served as the positive control. FRAP values (expressed as mg Fe (II)/g of the extract) for the extracts were then extrapolated from the standard curve.

Reducing Power Assay

The reducing power was determined according to the method described by Lai *et al.* (2001). One millilitre of different concentrations of extracts (0.1-1.0 mg/mL) in water was mixed with 2.5 mL of 0.2 M phosphate buffer, pH 6.6 and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min. Thereafter, 2.5 mL of trichloroacetic acid (10%) was added to the mixture to stop the reaction. Then 2.5 mL of distilled water and 0.5 mL of 0.1% FeCl₃ were added and the absorbance measured at 700 nm. Higher absorbance

values indicated higher reducing power. Vitamin C served as a positive control.

Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

TBARS was estimated according to the method of Ohkawa *et al.* (1979). Egg yolk homogenate (0.5 mL of 10% v/v) and 0.1 mL of extract were added to a test tube and made up to 1 mL with distilled water. 0.05 mL of FeSO₄ (0.07 M) was added to induce lipid peroxidation and incubated for 30 min at room temperature. Then 1.5 mL of 20% acetic acid (pH adjusted to 3.5 with NaOH) and 1.5 mL of 0.8% (w/v) TBA in 1.1% sodium dodecyl sulphate and 0.05 mL 20% TCA were added and the resulting mixture was vortex and then heated at 95 °C for 60 min. The generated colour was measured at 532 nm. Inhibition of lipid peroxidation (%) was calculated with formula:

$$(C-E)/C \times 100\%$$

where C is the absorbance value of the fully oxidized control and E is (Abs_{532+TBA} - Abs_{532-TBA}).

Statistical analysis

All analyses were carried out in triplicate and results expressed as mean ± SEM. The data were subjected to one-way analysis of variance (ANOVA), where applicable. Differences between means were determined by Duncan's multiple range tests using Graph Pad Prism statistical package version 6. P values of < 0.05 were regarded as significant.

RESULTS

Total phenolic contents are shown in Figure 1. Total phenolic content is reported as mg gallic acid equivalent/g of extract by

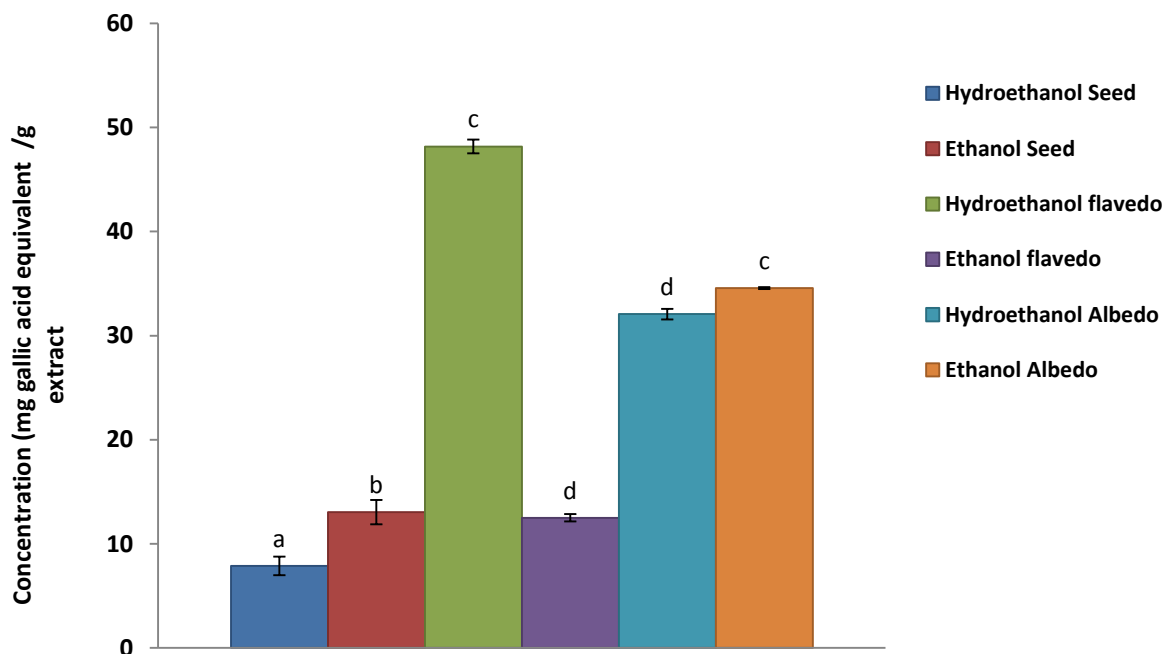


Figure 1:

Total phenolic content of hydroethanol and ethanol extracts from the seeds, flavedo and albedo of *Citrus sinensis* fruit. Different lower case letters represent significant difference between means at $p < 0.05$.

reference to a standard curve ($y = 0.003 + 0.077x$, $R^2 = 0.939$). Total phenolic contents were higher in the ethanol seed and albedo extracts (13.05 ± 0.35 and 34.57 ± 0.09 mg gallic acid equivalent/g of extracts, respectively) compared to the hydroethanol extracts (7.88 ± 0.88 and 32.07 ± 0.67 mg gallic acid equivalent/g of extracts, respectively). The hydroethanol flavedo extract however contained significantly higher total phenolics (48.17 ± 1.17 mg gallic acid equivalent/g of extract) compared to the ethanol flavedo extract (12.50 ± 0.50 mg gallic acid equivalent/g of extract).

The total flavonoid content is reported as mg quercetin equivalent/g of extract by reference to a standard curve ($y = 0.007x - 0.041$; $R^2 = 0.970$). Total flavonoids showed the same trend for the seed and flavedo extracts (20.93 ± 0.07 and 45.79 ± 0.22 mg quercetin equivalent/g of extract, respectively for the hydroethanol extracts and 14.29 ± 0.38 and 39.93 ± 1.22 mg quercetin equivalent/g of extract for the ethanol extracts (figure 2). The total flavonoid contents for the hydroethanol and ethanol albedo extracts were 15.93 ± 0.93 and 23.43 ± 2.57 mg quercetin equivalent/g of extract, respectively.

The DPPH radical scavenging activities of the extracts are presented in figure 3. DPPH radical scavenging activity assay showed that the hydroethanol flavedo and seed extracts were the best inhibitors of the DPPH radical at higher concentrations (50 – 200 µg/mL) of the extracts. The hydroethanol flavedo extract was observed to have a dose-dependent increase in % inhibition as concentration increased. IC₅₀ values for all extracts were found to be significantly higher than that of the reference antioxidant, ascorbic acid (0.06 ± 0.01 mg/mL). IC₅₀ values ranged from 0.18 ± 0.01 mg/mL in the hydroethanol seed extract to 0.34 ± 0.02 mg/mL in the ethanol seed extract (table 1).

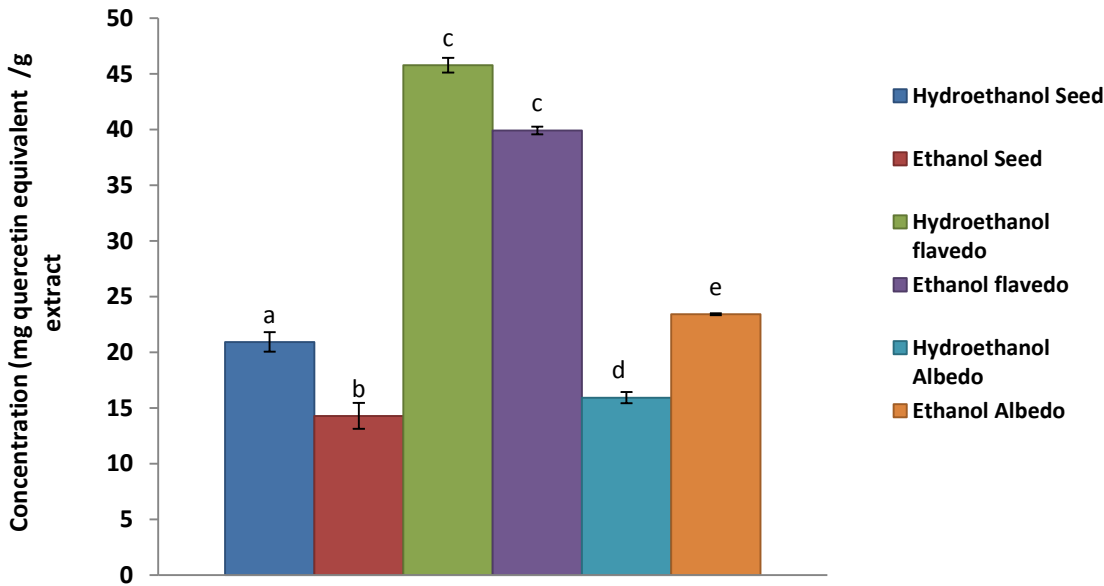


Figure 2: Total flavonoid content of hydroethanol and ethanol extracts from the seeds, flavedo and albedo of *Citrus sinensis* fruit. Different lower case letters represent significant difference between means at $p < 0.05$.

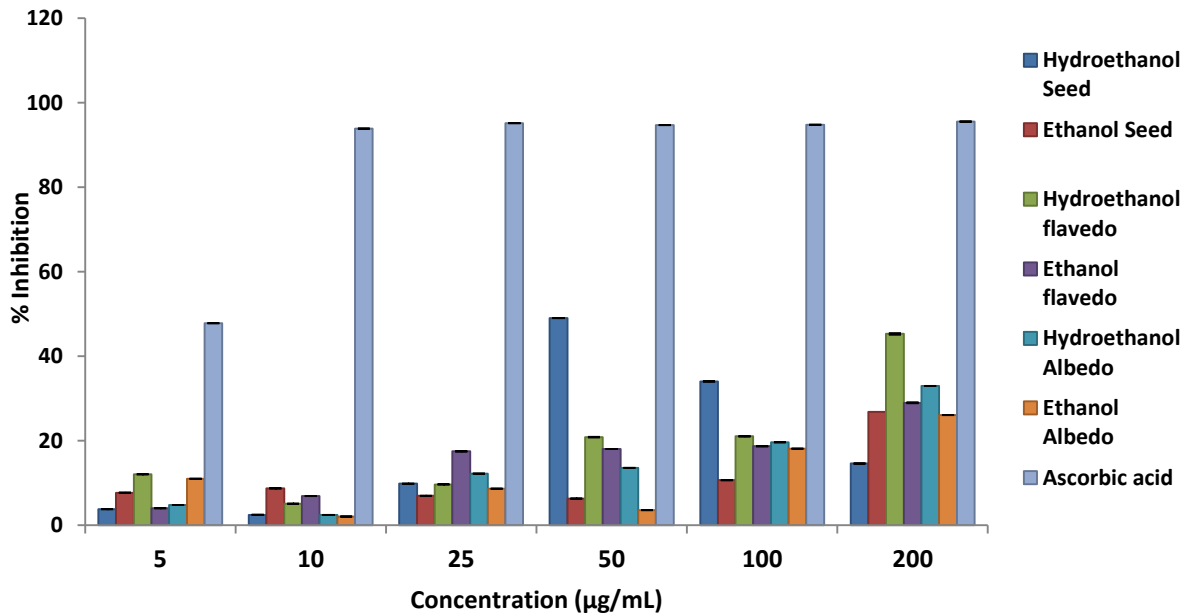


Figure 3: Graph comparing the DPPH's radical scavenging activities of different concentrations of ascorbic acid, and extracts from the seeds, flavedo and albedo of *Citrus sinensis* fruit

The ferric reducing antioxidant potential (FRAP) values for the hydroethanol flavedo extract ($800.30 \pm 1.53 \mu\text{mole Fe(II)/g extract}$) was found to be significantly higher ($P < 0.05$) than that of the standard ascorbic acid ($770.75 \pm 1.75 \mu\text{mole Fe(II)/g extract}$) (figure 4). All other extracts contained significantly lower FRAP values than the standard ascorbic acid. It was generally observed that the hydroethanol extracts contained higher FRAP values in each fruit material compared to the corresponding ethanol extracts. The seed extracts contained the lowest FRAP values (329.00 ± 1.53 and $464.30 \pm 0.75 \mu\text{mole Fe(II)/g extract}$ for ethanol and hydroethanol

extracts respectively). The flavedo extracts were observed to contain the highest FRAP values (800.30 ± 1.53 and $599.30 \pm 1.17 \mu\text{mole Fe(II)/g extract}$ for hydroethanol and ethanol extracts, respectively) with the albedo extracts containing 708.75 ± 1.75 and $507.30 \pm 1.61 \mu\text{mole Fe(II)/g extract}$ for hydroethanol and ethanol extracts, respectively.

The reducing power of all the extracts were significantly lower ($p < 0.05$) than that of the standard ascorbic acid at concentrations of $0.2 - 0.8 \text{ mg/mL}$ (figure 5). The hydroethanol extracts were also found to generally contain higher reducing powers than the ethanol extracts. The

hydroethanol extract of the flavedo had the highest reducing powers at the concentrations studied followed by the albedo extract.

Figure 6 shows the inhibition of lipid peroxidation as estimated by the thiobarbituric acid reactive substances

The DPPH radical scavenging activities of the extracts are presented in figure 3. DPPH radical scavenging activity assay showed that the hydroethanol flavedo and seed extracts were the best inhibitors of the DPPH radical at higher concentrations (50 – 200 µg/mL) of the extracts. The hydroethanol flavedo extract was observed to have a dose-dependent increase in % inhibition as concentration increased. IC₅₀ values for all extracts were found to be significantly higher than that of the reference antioxidant, ascorbic acid (0.06 ± 0.01 mg/mL). IC₅₀ values ranged from 0.18 ± 0.01 mg/mL in the hydroethanol seed extract to 0.34 ± 0.02 mg/mL in the ethanol seed extract (table 1).

Table 1: IC₅₀ values for *Citrus sinensis* fruit waste extracts.

Sample	IC ₅₀ value (mg/mL)
Ascorbic acid	0.06 ± 0.01 ^a
Hydroethanol seed	0.18 ± 0.01 ^b
Ethanol seed	0.34 ± 0.02 ^c
Hydroethanol flavedo	0.22 ± 0.01 ^d
Ethanol flavedo	0.26 ± 0.03 ^e
Hydroethanol albedo	0.23 ± 0.01 ^d
Ethanol albedo	0.27 ± 0.02 ^e

Data represent mean ± SEM of triplicate analysis. Different lowercase letters within column indicate significant difference at p ≤ 0.05.

The ferric reducing antioxidant potential (FRAP) values for the hydroethanol flavedo extract (800.30 ± 1.53 µmole

(TBARS) test. Inhibition of lipid peroxidation was highest in the ethanol flavedo extract (29.46 ± 0.08 %) and lowest in the ethanol seed extract (8.46 ± 0.15%).

Fe(II)/g extract) was found to be significantly higher (P < 0.05) than that of the standard ascorbic acid (770.75 ± 1.75 µmole Fe(II)/g extract) (figure 4). All other extracts contained significantly lower FRAP values than the standard ascorbic acid. It was generally observed that the hydroethanol extracts contained higher FRAP values in each fruit material compared to the corresponding ethanol extracts. The seed extracts contained the lowest FRAP values (329.00 ± 1.53 and 464.30 ± 0.75 µmole Fe(II)/g extract for ethanol and hydroethanol extracts respectively). The flavedo extracts were observed to contain the highest FRAP values (800.30 ± 1.53 and 599.30 ± 1.17 µmole Fe(II)/g extract for hydroethanol and ethanol extracts, respectively) with the albedo extracts containing 708.75 ± 1.75 and 507.30 ± 1.61 µmole Fe(II)/g extract for hydroethanol and ethanol extracts, respectively.

The reducing power of all the extracts were significantly lower (p < 0.05) than that of the standard ascorbic acid at concentrations of 0.2 - 0.8 mg/mL (figure 5). The hydroethanol extracts were also found to generally contain higher reducing powers than the ethanol extracts. The hydroethanol extract of the flavedo had the highest reducing powers at the concentrations studied followed by the albedo extract.

Figure 6 shows the inhibition of lipid peroxidation as estimated by the thiobarbituric acid reactive substances (TBARS) test. Inhibition of lipid peroxidation was highest in the ethanol flavedo extract (29.46 ± 0.08 %) and lowest in the ethanol seed extract (8.46 ± 0.15%)

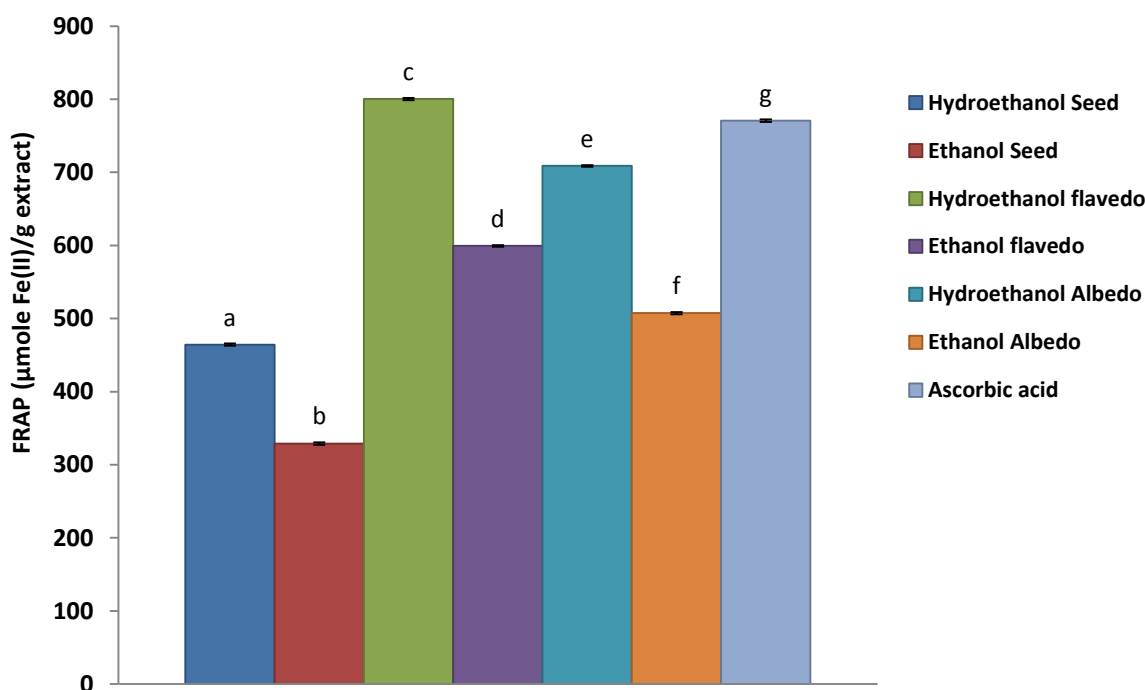


Figure 4: Ferric reducing antioxidant potential (FRAP) of *Citrus sinensis* fruit extracts. Different lower case letters represent significant difference between means at P < 0.05.

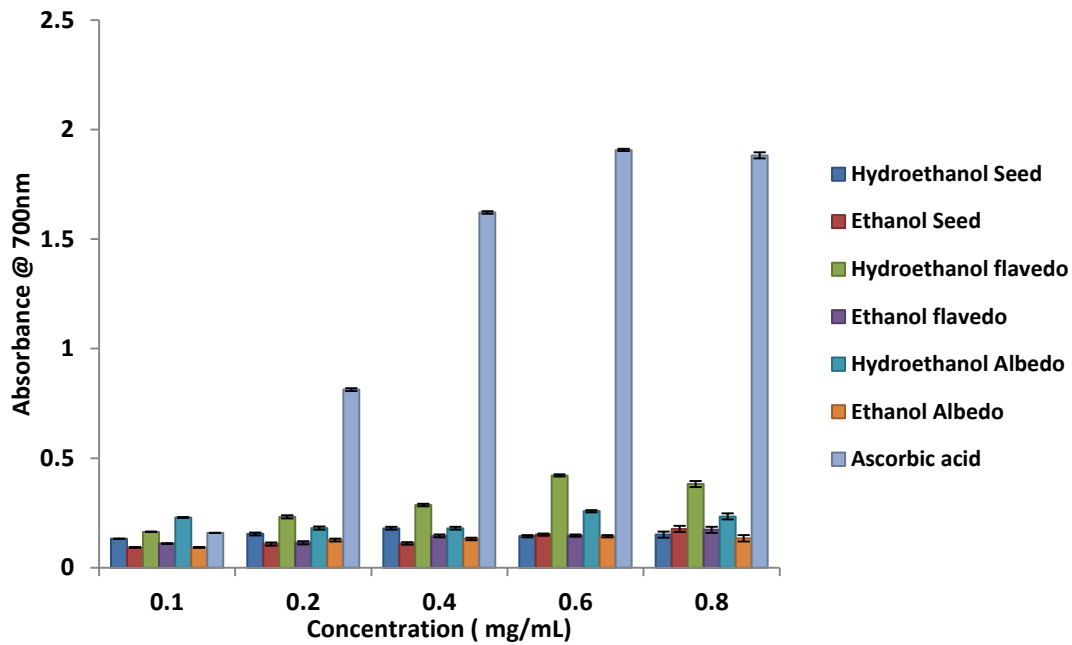


Figure 5: Reducing potential of both hydroethanol and ethanol extracts from the seeds, flavedo and albedo of *Citrus sinensis* fruit

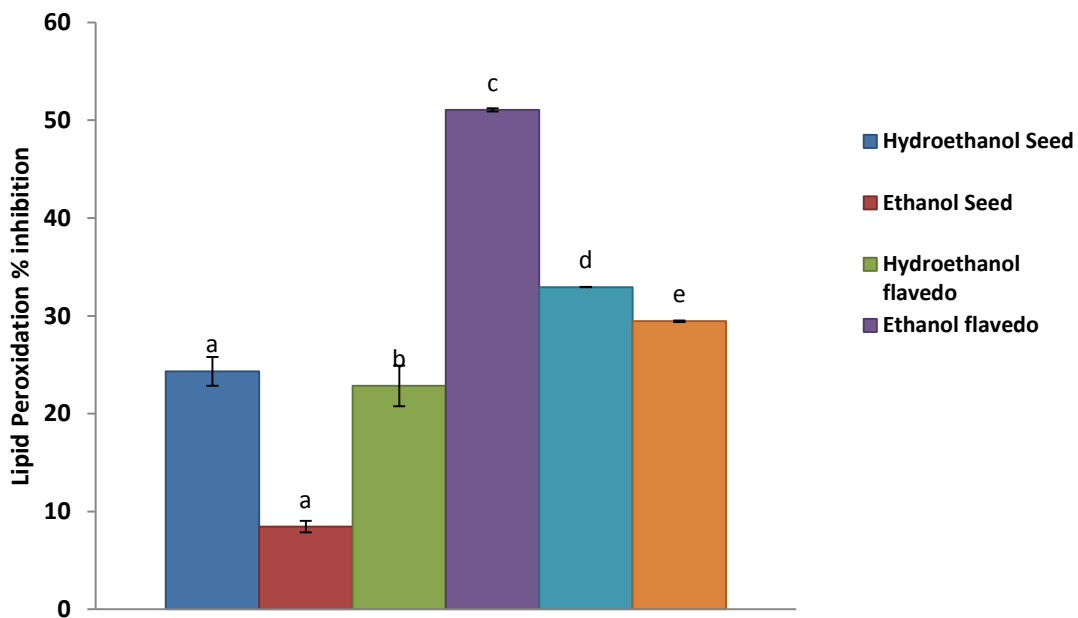


Figure 6: Thiobarbituric acid reactive substances of hydroethanol and ethanol extracts from seeds, flavedo and albedo of *Citrus sinensis* fruit

DISCUSSION

It has been suggested that fruits, vegetables and plants are the main source of antioxidant in the diet. Natural antioxidants may act as free-radical scavengers, reducing agents, complexes of pro-oxidant metals, quenchers of singlet oxygen etc (Govindapa *et al.*, 2011). Fruits and vegetables are known to confer protection against oxidative stress in several diseases. This protective effect is attributable to the presence of several antioxidants and vitamins in them (Gudha *et al.*, 2011).

The use of different solvents for extraction process is advisable as natural antioxidants are multifunctional and different solvents may preferentially extract different antioxidant

molecules (Gan *et al.*, 2013). Ferrari *et al.*, (2012) observed that hydroethanol extracts produced the best yields which are also acceptable for human consumption. Indeed, results from this study demonstrate that ethanol and hydroethanol extracts of the same fruit waste material possess different phenolic constituents and antioxidative capacities.

Polyphenols are known to possess good antioxidative activities. This is because plant polyphenols donate electrons or hydrogen atoms to terminate radical chain reactions by converting free radicals to more stable products, therefore exhibiting strong antioxidant activity (Ayoola *et al.*, 2011). The results of this study showed that phenolics were highest in the hydroethanolic flavedo extract followed by both

albedo extracts. The seed extracts had the least phenolic content.

Flavonoids are a ubiquitous group of polyphenolic substances present in most plants. They are known to exhibit diverse medicinal activities and are hence valuable in the prevention and treatment of a number of diseases (Ayoola *et al.*, 2008). Flavonoids were highest in both flavedo extracts.

The DPPH free radical scavenging assay is a rapid and sensitive method for estimating antioxidant activity in plant extracts (Sirag *et al.*, 2014). The DPPH test shows the ability of the test compound to act as a free radical scavenger. DPPH is a free radical and it gives a strong absorption band at 517nm in the visible region of the electromagnetic spectrum. It has a deep violet colour. This absorption diminishes as the electron is paired off resulting in decolorization with respect to the number of electrons taken up and the colour changes to a pale yellow. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The more antioxidants occur in an extract, the more DPPH reduction occurs (Ayoola *et al.*, 2008; Rahman *et al.*, 2013). IC₅₀ value is negatively related to antioxidant activity as it expresses the amount of antioxidant needed to decrease the radical concentration by 50%. The lower the IC₅₀ value, the higher the antioxidant activity of the tested sample (Chanda *et al.*, 2011).

The IC₅₀ values for the extracts showed that all extracts had significantly higher IC₅₀ values than the reference antioxidant, vitamin C. Amongst the extracts, the hydroethanol seed extract had the lowest IC₅₀ values (0.18 ± 0.01 mg/mL) suggesting that this extract had the best DPPH radical scavenging ability.

Different methods are utilized in the determination of antioxidant capacity of plant materials. This is because differences exist between them in terms of their assay principles and experimental conditions (Katalinic *et al.*, 2006). DPPH assay procedure reflects only the activity of water-soluble antioxidants. FRAP assay is a simple, fast and precise assay that measures the total antioxidant power of biological fluids (Aljadi and Kamaruddin, 2004). FRAP assay is reproducible and linearly related to the molar concentration of the antioxidant(s) present in the biological sample. It was initially developed to assay plasma antioxidant capacity but however is currently used in a range of biological samples such as fruits, wines and animal tissues (Katalinic *et al.*, 2006). The hydroethanol flavedo extract compares favourably with ascorbic acid as it has a higher FRAP value (800.30 ± 1.53 µmole Fe(II)/g extract) than ascorbic acid (770.75 ± 1.75 µmole Fe(II)/g extract). The hydroethanol albedo extract also has good reducing potential (708.75 ± 1.75 µmole Fe(II)/g extract).

The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity because the presence of reductants such as antioxidant substances in the sample causes the reduction of the Fe³⁺-ferricyanide complex to the ferrous form (Rahman *et al.*, 2013). Apart from FRAP assay, reducing power provides more insight into the reductive capacity and hence, antioxidant activity of a biological sample. Reducing power assay method is based on the principle that substances which have reduction potential, react with potassium ferricyanide (Fe³⁺) to form potassium ferrocyanide (Fe²⁺), which then reacts with ferric chloride to form ferric ferrous complex that has an absorption maximum at 700 nm (Talukder *et al.*, 2013). The results of the reducing power assay essentially follows the same trend as the FRAP assay. The hydroethanol flavedo and albedo extracts

contain the highest reducing power as evidenced from their having the highest absorbance values. The ethanol flavedo and albedo extracts are also observed to contain higher absorbance values than the seed extracts and hence, possess higher reducing powers. This may be due to the higher phenolic and flavonoid contents observed in the flavedo and albedo extracts compared to the seed extracts.

The reproducible results from the FRAP and reducing power assays suggest that either test may be used alone in the absence of the other to confirm the reductive potential of an extract. Oxidative stress results in the damage of biopolymers including nucleic acids, proteins, polyunsaturated fatty acids and carbohydrates (Mogana *et al.*, 2013). Lipid peroxidation occurs as a result of oxidative deterioration of polyunsaturated lipids and it involves ROS and transition metal ions. It is a molecular mechanism of cell injury that yields a wide range of cytotoxic products, most of which are aldehydes, like malondialdehyde (MDA), 4-hydroxynonanal (HNE) (Badarinath *et al.*, 2010). *In vitro* inhibition of lipid peroxidation therefore is another index for evaluating the antioxidative capacity of an extract. Our results show that the ethanol flavedo extract is a good inhibitor of lipid peroxidation (51.06±0.02 %). Aside the ethanol seed extract with % inhibition of 8.46±0.15 %, all the other extracts inhibit lipid peroxidation at percentages ranging from 22.85±0.59 % to 32.94±2.08 %.

In conclusion, the results of this study have demonstrated that the hydroethanol extracts had better antioxidative activities when compared to the ethanol extracts. Hydroethanol may therefore be a better solvent for extracting important compounds with antioxidative properties from sweet orange.

The hydroethanol flavedo extract contained the highest amounts of phenolics and had the best antioxidative activities of all extracts tested. The superior antioxidant activity observed in this extract may be linked to the abundant levels of phytochemicals detected in this extract in our previous study (Oikeh *et al.*, 2013).

Consumers of sweet orange (*Citrus sinensis*) commonly utilize only the juicy pulp while discarding the flavedo, seeds and albedo components. Industrial fruit juice processing companies also consider the flavedo, seeds and albedo to be a nuisance in the production process. This paper has demonstrated that these “useless” components can be further utilized to obtain products with potential pharmacological action. While some researchers have previously evaluated antioxidative activity in Citrus peels, this study also draws attention to the potential usefulness of the albedo and seed components.

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