

# A comparative study on antioxidant properties, proximate and mineral compositions of the peel and pulp of ripe *Annona muricata* (L.) fruit

\*Akomolafe, S.F. and Ajayi, O.B.

Department of Biochemistry, Ekiti State University, P.M.B 5363, Ekiti - State, Nigeria

#### Article history

## Abstract

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#### **Keywords**

Soursop Peel Pulp Phytochemicals Mineral Proximate The leaves, seeds and roots of Annona muricata (L.) (soursop) have been studied for pharmaceutical purposes with little attention paid to the study of the pulp and peel of its fruit. This study was therefore conducted to compare the antioxidant properties, phytocontituents, proximate and mineral compositions of the peel and pulp of ripe A. muricata fruit. The percentage protein, ash and fibre contents were higher in the peel while percentage fat, moisture, carbohydrate contents and total gross energy were higher in the pulp than the peel. The K/ Na ratio of the peel and pulp were higher than 1.0 recommended. The antioxidant potentials measured by ferric reducing antioxidant power (FRAP), 1,1-diphenyl-2-picrylhydrazyl (DPPH), Fe<sup>2+</sup> chelation and hydroxyl radical scavenging ability tests in soursop peel were significantly higher than in the pulp. The peel also showed the strongest inhibition of lipid peroxidation. Furthermore, the result revealed the highest phenolic, flavonoid and vitamin C contents in the soursop peel. Free radical scavenging potentials of the extracts were found to be proportional to their respective phenolic and flavonoid contents. The results of our present study indicate that soursop peel exhibited significantly higher mineral composition, proximate composition, antioxidant activity and phytoconstituents compared to the pulp and thus, being an agrowaste, can be explored as a viable source of natural antioxidants for the functional food and pharmaceutical applications.

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# Introduction

Reactive oxygen species (ROS) and reactive nitrogen species (RON) are free radicals, which derived either from normal metabolic processes or from external sources (Magalhaes, 2006). These species are essential to energy supply, detoxification, chemical signalling and immune function (Dimitrios, 2006). However, overproduction of these free radical may lead to damage to valuable biomolecules such as DNA, lipids and proteins. These free radicals are often associated with the oxidation in food and biological systems which will cause oxidative rancidity in foods and the development of several human diseases such as neurological degeneration, diabetes and certain types of cancer (Magalhaes, 2006).

An antioxidant is any substance which is capable of delaying, preventing the oxidative damage of lipids, protein and nucleic acids by reactive oxygen species, which include reactive free radicals such as superoxide, hydroxyl, peroxyl, alkoxyl and nonradicals such as hydrogen peroxide and hypochlorous (Lim *et al.*, 2007). Thus, antioxidants have gained numerous attention in the past few years, especially within the food, biological and agrochemical fields.

There is increasing evidence that the consumption

of vegetables and fruits is associated with a reduced risk of degenerative disease such as cancer, cardiovascular disease and cataracts (Chinnici et *al.*, 2004). This association is often attributed to the natural antioxidant present in fruits and vegetables, such as vitamin C and E, carotenoids, phenolic acids and flavonoids, which prevent free radical damage (Silva *et al.*, 2004). Epidemiological studies have established a positive correlation between the intake of fruits and vegetables and prevention of diseases like atherosclerosis, cancer, diabetes, arthritis and also ageing (Kaur and Kapoor, 2001)

Annona muricata L. (A. muricata) is one of the tropical fruits that demonstrate antioxidant properties. This plant contains annonaceous acetogenins in the twigs, unripe fruit, seeds, roots, and bark tissues, which display antitumor, pesticidal, antimalarial, anthelmintic, piscicidal, antiviral, and antimicrobial effects, thus suggesting many potentially useful applications. Ripe A. muricata pulp extract contains three prominent acetogenins: asimicin, bullatacin, and bullatalicin. Previous research on A. muricata was focused on the leaves, seeds and roots for pharmaceutical purposes (Gleeve et al., 1997; Jaramillo et al., 2000; Onimawo, 2002). Little attention has been paid to the study of the pulp and peel of *A. muricata* fruit. This study was therefore conducted to compare the antioxidant properties, phytocontituents, proximate and mineral compositions of the peel and pulp of *A. muricata*.

# **Materials and Methods**

#### Annona muricata L. extract

Fresh ripe sample of *A. muricata* fruit was gotten from a local farm in Ado - Ekiti metropolis, Ado west local government area and identified in the herbarium of the Department of plant science, Ekiti State University by Mr Ajayi where voucher specimen (number UHAE 338) was deposited in the herbarium of the same Department. The fresh extract was prepared by blending the fruit pulp (without the seeds) and peel separately with the ratio of 1:5 (peel or pulp: distilled water) by using a warring blender. The mixture was filtered by vacuum filtration. The filtrate was then concentrated in a rotary evaporator at 4°C before being used for the analyses.

## Animal

All procedures concerning the use of animals were approved by the Animal Ethic Committee of Ekiti State University, Ado Ekiti, Nigeria. The animals were housed in a controlled environment, with room temperature (37°C) and a relative humidity of 30-70%. The rooms were illuminated with 12 hours artificial fluorescent light and 12 hours darkness per day. The animals were provided with a standard pelleted and distilled water via ad libitum.

## Chemicals and reagents

Thiobarbituric acid (TBA), 1,10-phenanthroline, deoxyribose, gallic acid, and Folin-Ciocalteau's reagent were procured from Sigma-Aldrich, Inc., (St. Louis, MO, USA), trichloroacetic acid (TCA) was sourced from Sigma-Aldrich, Chemie GmbH (Steinheim, Germany), dinitrophenyl hydrazine (DNPH) from ACROS Organics (NJ, USA), hydrogen peroxide, methanol, acetic acid, and FeCl3 were sourced from BDH Chemicals Ltd., (Poole, England), thiourea, CuSO<sub>4</sub>·5H<sub>2</sub>O, H<sub>2</sub>SO<sub>4</sub>, sodium carbonate, AlCl3, potassium acetate, Tris-HCl buffer, sodium dodecyl sulphate, FeSO<sub>4</sub>, and potassiumferricyanide were of analytical grade.

# Determination of total phenolic content

The total phenolic content was determined in the extracts using the method of Singleton *et al.* (1999). 250  $\mu$ L of the extracts were oxidized with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v) and neutralized by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 minutes at 45°C and the absorbance was measured at 765 nm in the spectrophotometer (JENWAY 6305, Barloworld Scientific, Dun- mow, United Kingdom). The total phenolic content was subsequently calculated as gallic acid equivalent.

#### Determination of total flavonoid content

The total flavonoid contents of both extracts (peel and pulp) were determined using a slightly modified method of Meda *et al.* (2005). Briefly, 0.5 mL of the sample was mixed with 0.5 mL methanol, 50  $\mu$ L 10% AlCl<sub>3</sub>, 50  $\mu$ L of 1 M Potassium acetate and 1.4 mL of distill water, and allowed to incubate at room temperature (37°C) for 30 minutes. The absorbance of the reaction mixture was subsequently measured at 415 nm and the total flavonoid content was subsequently calculated as quercetin equivalent.

## Determination of vitamin C content

The vitamin C content of the two extracts was determined using the method of Benderitter *et al.* (1998). Briefly, 75  $\mu$ L DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg CuSO<sub>4</sub>•5H<sub>2</sub>O in 100 mL of 5M H<sub>2</sub>SO<sub>4</sub>) were added to 500  $\mu$ L reaction mixture (300  $\mu$ L of extract with 100  $\mu$ L 13.3% trichloroacetic acid (TCA) and water). The reaction mixtures were subsequently incubated for 3 hours at 37°C, then 0.5 mL of 65% H<sub>2</sub>SO<sub>4</sub> was added to the medium. The absorbance of the reaction mixture was measured at 520 nm. The vitamin C content of the samples was subsequently calculated as ascorbic acid equivalent.

#### Preparation of tissue homogenates

The rats were decapitated under mild diethyl ether anesthesia and the liver was rapidly dissected and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1/10) with about 10 strokes at approximately 1200 rev/minute in a Teflon glass homogenizer (Mexxcare, mc14 362, Aayu-shi Design Pvt. Ltd., India). The homogenate was centrifuged (KX3400C Kenxin Intl. Co. Hong Kong) for 10 minutes at 3000 × g to yield a pellet that was discarded, and a low-speed supernatant, which was kept for lipid peroxidation assay (Belle *et al.*, 2004).

# Lipid peroxidation and thiobarbibutric acid reactions

The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al.* (1979). Briefly, 100  $\mu$ L of the SI fraction was mixed with a reaction mixture containing 30  $\mu$ L of 0.1M pH 7.4 Tris- HCl buffer, extract (0 - 100  $\mu$ L), and 30  $\mu$ L of

250  $\mu$ M freshly prepared FeSO<sub>4</sub> as the pro-oxidant. The volume was made up to 300  $\mu$ L by water before incubation at 37°C for 2 hours. The colour reaction was developed by adding 300  $\mu$ L 8.1% SDS (sodium dodecyl sulphate) to the reaction mixture, this was subsequently followed by the addition of 500  $\mu$ L of acetic acid/HCl (pH 3.4) mixture and 500  $\mu$ L of 0.8% thiobarbituric acid (TBA). This mixture was incubated at 100°C for 1 hour. Thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm and expressed as (%) malondialdehyde (MDA) produced using MDA standard curve (0–0.035mM).

# DPPH free radical scavenging ability

The free radical scavenging ability of the extracts against 1,1-diphenyl-2 picrylhydrazyl (DPPH) free radical was evaluated as described by Gyamfi *et al.* (1999). Briefly, an appropriate dilution of the extract (1 mL) was mixed with 1 mL of 0.4 mM methanolic solution containing DPPH radicals, the mixture was left in the dark for 30 minutes and the absorbance was measured at 516 nm. The control was carried out using 2 mL DPPH solution without the test samples. The DPPH free radical scavenging ability was subsequently calculated thus: DPPH scavenging ability (%) = [(absorbance of control – absorbance of samples) / absorbance of control] × 100.

# $Fe^{2+}$ -chelation assay

The Fe<sup>2+</sup> chelating ability of both extracts was determined using a modified method of Puntel et al. (2005). Freshly prepared 500  $\mu$ M FeSO<sub>4</sub> (150  $\mu$ L) was added to a reaction mixture containing 168  $\mu$ L of 0.1M Tris-HCl (pH 7.4), 218  $\mu$ L saline and the extract. The reaction mixture was incubated for 5 minutes before the addition of 13  $\mu$ L of 0.25% 1,10-phenanthroline. The absorbance was subsequently measured at 510 nm. The Fe (II) chelating ability was subsequently calculated with respect to the reference (which contains all the reagents without the test sample).

# Determination of reducing property

The reducing property of the extracts was determined by assessing the ability of the extracts to reduce FeCl<sub>3</sub> solution as described by Oyaizu (1986). A 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 minutes and 2.5 mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 minutes. 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was

measured at 700 nm. The ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

# Proximate analysis

Moisture, total ash, fiber and ether extract of the samples were determined by the methods of the AOAC (2005). Nitrogen was determined by a micro-Kjeldahl method and the crude protein content was calculated as N x 6.25 (Pearson, 1976). Carbohydrate was determined by difference. All the proximate results were reported in g/100 g dry weight. The energy values obtained for carbohydrates (x 17 kJ), crude protein (x 17 kJ) and crude fat (x 37 kJ) for each of the samples. Determinations were in duplicate.

# Mineral analysis

The mineral elements were determined in the solutions as follows-Na and K by flame photometry, Model 405 (Corning, Halstead, Essex, UK) using NaCl and KCl to prepare standards. Minerals were analyzed using the solutions obtained by dry ashing the samples at 550°C and dissolving it in 10% HCl (25 ml) and 5% lanthanum chloride (2 ml), boiling, filtering and making up to standard volume with deionized water. Phosphorus was determined colorimetrically using a Spectronic 20 (Gallenkamp, London, UK) instrument, with  $KH_2PO_4$  as a standard. All other elements (Ca, Mg and Zn) were determined by atomic absorption spectrophotometry, Model 403 (Perkin-Elmer, Norwalk, Connecticut, USA). All determinations were made in duplicate. All chemicals used were of analytical grade, and were obtained from the British Drug House (BDH, London, UK).

The detection limits for the metals in aqueous solution had been determined just before the mineral analyses using the methods of Varian Techtron, giving the following values in  $\mu g/ml$ : Na (0.002), K (0.005), Ca (0.04), Mg (0.002) and Zn (0.005) (Varian Techtron, 1975). The optimal analytical range was 0.1 to 0.5 absorbance units with coefficients of variation from 0.9-2.2%. The coefficients of variation per cent were calculated (Steel and Torrie, 1960). The percentage contribution to energy due to protein (PEP), due to total fat (PEF) and due to carbohydrate (PEC) as PEP%, PEF% and PEC% respectively were calculated. The percentage utilizable energy due to protein (UEDP %) was also calculated. Ca/P, Na/K, Ca/Mg and the millequivalent ratio of [K/(Ca + Mg)]; the mineral safety index (MSI) of Na, Mg, P, Ca and Zn were also calculated (Hathcock, 1985). To calculate MSI, we have: RAI is recommended adult intake; CV in the Table will represent calculated value (CV) of calculated MSI from research results.

Parameters	peel	pulp
Crude protein	$15.62\pm0.01$	$1.38\pm0.01$
Crude fat	$6.46\pm0.02$	$9.75\pm0.01$
Total ash	$1.99\pm0.01$	$0.61\pm0.00$
Crude fibre	$8.21\pm0.01$	$3.02\pm0.01$
Moisture	$13.26\pm0.06$	$18.10\pm0.06$
Carbohydrate %	$54.46\pm0.22$	$67.14\pm0.09$
PEC %	64.72	74.82
PEF %	16.71	23.65
PEP %	18.56	1.54
UEDP %	11.14	0.92
Energy kJ/100g	1430	1526

Table 1. Proximate composition (mg/g) of the peel and pulp of *Annona muricata* (L.) fruit (on dry weight basis)

PEP = Proportion of total energy due to protein

PEF = Proportion of total energy due to fat

PEC = Proportion of total energy due to carbohydrate

UEDP = Utilizable energy due to protein

The differences between the standard MSI and the MSI of the samples were also calculated.

# Data analysis

The results of replicate readings were pooled and expressed as mean  $\pm$  standard deviation. One way analysis of variance was used to analyze the results and Duncan multiple test was used for the post hoc (Zar, 1984). Statistical package for Social Science (SPSS) 16.0 for Windows was used for the analysis. The significance level was taken at P<0.05.

#### **Results and Discussion**

In this study, the peel and pulp of *A. muricata* fruit has low moisture content, but the value obtained for pulp was higher than that of the peel (Table 1). The low moisture content of the soursop pulp and its peel could also have affected some of the value of other nutrients present. The moisture content obtained in this study was lower than that of conventional fruits such as *Citrus sinensis* fruit (75%) and *Citrus lanatus* fruit (89%) (Ozioma, 2013) but higher than pineapple (0.86%) and banana (0.03%) (Harbourne, 1983).

The higher ash value of soursop peel (1.99%) compared to that of soursop pulp (0.61%) indicated that the peels are good sources of minerals and therefore can be used in diet supplementation which will improve the mineral quality of diets. These minerals act as inorganic co-factors in

metabolic processes which mean in the absence of these inorganic co-factors, there could be impaired metabolism (Iheanacho and Udebuani, 2009). As obtained from the result of this study, soursop pulp contains a higher content of fats than the peel, this result was in contrast to the report of Onyechi (2012) that reported no fat content in the same fruit. The high protein content of the soursop peel than that of the pulp is an indication that the soursop peel had concentrated of protein than the pulp (Table 1). The daily energy requirement for an adult is between 2500-3000 kCal (10455-12548 kJ) depending on his physiological state while that of infants is 740 kCal (3094.68 kJ) (Bingham, 1978). This implies that while an adult man would require between 599-720 g from the peel (taking the calculated energy of 1430 kJ/100 g) of his energy requirement, then it would require 562-675 g from the pulp (taking the calculated energy of 1430 kJ/100 g), whereas infants would require 177.8 g from the peel while requiring 166.6 g from the pulp. On the whole this meant that samples with higher energy value would require a lower quantity of sample to satisfy the energy needs of man and infants. The utilizable energy due to protein (UEDP%) of the peel compared favourably with the recommended safe level of 8% for an adult man who requires about 55 g protein per day with 60% utilization while that of pulp was very low (Table 1). The PEF% values were generally low in the two samples (Table 1) and far below the recommended

Parameters	peel	pulp						
Phosphorus (P)	$1.80\pm0.29$	$0.59\pm0.08$						
Calcium (Ca)	$0.60\pm0.14$	$0.61\pm0.07$						
Magnesium (Mg)	$0.17\pm0.07$	$0.05\pm0.01$						
Sodium (Na)	$1.06 \pm 0.03$	$0.37 \pm 0.13$						
Potassium (K)	$1.60 \pm 0.42$	$0.48\pm0.08$						
Zinc (Zn)	$0.38\pm0.18$	$0.30 \pm 0.28$						
K/Na	1.51	1.30						
Na/K.	0.66	0.77						
Ca/P	0.33	1.03						
Ca/Mg	3.53	12.20						
$[K/(Ca+Mg)]^a$	2.08	0.73						
Total phenolic (mg GAE/g)	$1.87\pm0.01^{a}$	$1.54\pm0.10^{\rm c}$						
Total flavonoid (mg QUE/g)	$0.54\pm0.00^{\text{b}}$	$0.26\pm0.00^{a}$						
Vitamin C (mg AAE/g)	$19.29\pm0.10^{\texttt{a}}$	$10.45\pm0.11^{b}$						

Table 2. Mineral composition (mg/g) and phytoconstituents of the peel and pulp of Annona muricata (L.) fruit

a = milliequivalent, AAE, ascorbic acid equivalent; GAE, gallic acid equivalent; QUE, quercetin equivalent; values represent mean  $\pm$  standard deviation of triplicate readings. values with the same superscript letter along the same row are not significantly (p  $\leq$  0.05) different.

level of 35% (COMA, 1984) for total fat intake; this is useful for people wishing to adopt the guidelines for a healthy diet.

Minerals are important in human nutrition. It is well known that enzymatic activities as well as the electrolytic balance of the blood fluid are related to the adequacy of Na, K, Mg and Zn. Potassium is very important in maintaining the blood fluid volume and osmotic equilibrium. Metal deficiency syndrome like rickets and calcification of bone is caused the deficiency. The levels of all the essential minerals were higher in the peel than in the pulp (Table 2). The peel was apparently high in phosphorus  $(1.8 \pm 0.29)$ mg/100g) while the pulp was high in calcium (0.61)  $\pm$  0.07 mg/100g). The Ca/P of the peel (Table 2) was comparably lower than 0.5 while that of pulp twice the value 0.5 which is the minimum ratio required for favourable calcium absorption in the intestine for bone formation (Nieman et al., 1992) although the level of Ca/P has been reported to have some effects on calcium in the blood of many animals (Adeyeye et al., 2012). The value of ratio Na/K of both the peel and pulp compared appreciably with 0.6 (Table 2), the value that favours non-enhancement of high blood pressure disease in man. Although for normal retention of protein during growth and for balancing fluid a K/Na ratio of 1.0 is recommended (Helsper et al., 1993), the high value of the K / Na ratio obtained in the present, report for both peel and pulp suggests that bringing the ratio down would require the consumption of food sources rich in no. The Ca/Mg value obtained from the peel and pulp were higher than the 1.0 recommended. It means both that both Ca and Mg would need adjustment for normal health.

The milliequivalent ratio [K/(Ca+Mg)] of the peel and pulp were comparably lower than 2.2 recommended, which means the sample would not promote hypomagesaemia in man (Adeyeye and Adesina, 2012). Zinc is among the required elements for humans and their daily requirements for adults is 15. Levels obtained in the present report (38 mg/100g (peel) and 30 mg/100g (pulp)) (Table 2) doubled the daily requirements, but the peel had the highest value. However, zinc requirements can easily be met by consuming this fruit.

There were also the presence of total phenolic, flavonoid and vitamin C in the soursop peel and pulp. This suggests that consumption of soursop could provide a lot of health benefits. Phenolic substances are pharmacologically active components of plants which are capable of neutralizing free radicals, chelating metal catalysts and inhibiting the activity of oxidizing enzymes in biological systems (Foti *et al.*, 1996; Dastmalchi *et al.*, 2007). They are also capable of regenerating endogenous  $\alpha$ - tocopherol in the phospholipid bilayer of the membrane to its active antioxidant form. This mechanism of antioxidant action confers health beneficial potentials on phenolic

Concentration (mg/ml)	Chelating ability		OH radical scavenging ability		Ferric reducing antioxidant property	
	peel	pulp	peel	pulp	peel	pulp
0.4	$13.65 \pm 2.40$	7.89 ± 1.19	$30.63 \pm 0.02$	8.84 ± 1.50	$3.32 \pm 1.20$	$1.54\pm0.10$
0.6	$21.07 \pm 1.36$	$13.37 \pm 1.21$	$37.25\pm2.50$	$13.76\pm0.80$		
0.8	$25.13\pm1.20$	$15.60\pm1.50$	$50.85\pm1.10$	$28.58 \pm 1.72$		
1.0	$38.70\pm0.90$	$19.76\pm1.95$	$68.66 \pm 0.50$	$37.45\pm0.02$		
1.2	48. 83 ± 2.20	$25.13\pm0.25$	$72.41 \pm 1.21$	$54.86\pm0.13$		

Table 3. Changes in the levels of chelating ability (%), OH radical scavenging ability (%) and ferric reducing antioxidant property (mmol.AAE/g) of the peel and pulp of of *Annona muricata* (L.) fruit

substances (Mccall and Frei, 1999; Louli *et al.*, 2004). The findings of our study revealed that soursop peel had the highest total phenolic, total flavonoid and vitamin C contents than the pulp (Table 3). The appreciable level of phenolic compounds in the soursop fruit, especially in the peel which are usually discarded by people could be useful for the treatment of radical related problems such as diabetes, ulcer and inflammation.

The ability of the soursop to reduce ferric ions to its ferrous form is evident in the formation of Perl's blue which was monitored spectrophotometrically at 700 nm. Our results revealed that the peel exhibited significantly higher antioxidant activity compared to the pulp (Table 3). Nevertheless, our study revealed that soursop is an electron donor that can react with free radicals to convert them into stable products and terminate the chain of reactions that leads to oxidative stress.

The peel and pulp of soursop chelate  $Fe^{2+}$  in a concentration dependent manner with peel being a better chelator than the pulp (Table 3). However, this result is in agreement with the  $FeSO_4$  -induced lipid peroxidation (Figure 2), phenolic content (Table 2) and antioxidant activity of the extracts, suggesting that  $Fe^{2+}$  chelation may be one of the possible mechanisms through which antioxidant phytochemicals from soursop prevent lipid peroxidation in tissue by forming a complex with  $Fe^{2+}$ , thus preventing the initiation of lipid peroxidation.

Hydroxyl radicals have been implicated in the oxidative damage of DNA, proteins and lipids (Mates, 2000; Walker and Everette, 2009). Among the reactive oxygen species, hydroxyl radicals are the most reactive and predominant radicals generated endogenously during aerobic metabolism to initiate cell damage *in vivo* (Spencer *et al.*, 1994). The peel and the pulp of soursop scavenged hydroxyl radicals in a concentration dependent manner. The scavenging ability of the peel was higher than the pulp (Table 3).



Figure 1. DPPH radical scavenging ability of the peel and pulp of *Annona muricata* (L.) fruit



Figure 2. Inhibitory effect of  $FeSO_4$  –induced lipid peroxidation in rat's liver by the peel and pulp of *Annona muricata* (L.) fruit

This observation suggests that the fruit, especially the peel can be used as an alternative remedy to synthetic antioxidants in combating the oxidative activity of hydroxyl radicals.

The trend of inhibition of DPPH radical by the peel and pulp of soursop were concentration dependent (Figure 1). The peel exhibited the highest inhibitory effect on DPPH radical which corresponds to its phenolic content. The strong inhibitory effect on DPPH radical of soursop could be linked to polyphenolic compounds which are capable of donating electrons to neutralize free radicals and thus, could be a promising therapeutic agent to treat stress induced pathological conditions.

The incubation of the liver homogenate in the presence of  $\text{FeSO}_4$  caused a significant increase in the MDA content when compared with the basal liver homogenate (Figure 2). The increase in the liver MDA content suggests lipid peroxidation. However, the peel and pulp of soursop inhibited MDA production in rat liver in a dose-dependent manner. Nevertheless, soursop peel had a significantly higher inhibitory effect on  $\text{FeSO}_4$ -induced lipid peroxidation in the liver homogenate than the soursop pulp (Figure 2). However, the reduced liver MDA content of the soursop peel and pulp in the rat liver may be a function of the antioxidant properties of the soursop fruit.

## Conclusion

The results of our present study indicate that soursop peel exhibited significantly higher mineral composition, proximate composition, antioxidant activity and phytoconstituents compared to the pulp and thus, being an agrowaste which are usually discarded by people, can be explored as a viable source of natural antioxidants for the functional food and pharmaceutical applications.

# References

- Adeyeye, E.I. and Adesina, A.J. 2012. Nutritional composition of African breadfruit (*Treculia africana*) seed testa. Journal Of Agricultural Research And Development 11(1): 159-178.
- AOAC 2005. International Official Methods of Analysis. 18<sup>th</sup> edn. Washington DC: Association of Analytical Chemists.
- Belle, N.A.V., Dalmolin, G.D., Fonini, G., Rubim, M.A. and Rocha, J.B.T. 2004. Polyamines Reduces Lipid Peroxidation Induced by Different Pro-Oxidant Agents. Brain Research 1008(2): 245-251.
- Benderitter, M., Maupoil, V., Vergely, C., Dalloz, F., Briot, F. and Rochette, L. 1998. Studies by Electron Paramagnetic Resonance of the Importance of Iron in Hydroxyl Scavenging Properties of Ascorbic Acid in Plasma Effects of Iron Chelators. Fundamental And Clinical Pharmacology 12(5): 510-516.
- Bingham, S. 1978. Nutrition: A consumer's guide to good eating. Transworld Publishers.London.
- Chinnici, F., Bendini, A., Gaiani, A. and Riponi, C. 2004. Radical scavenging activities of peels and pulp from cv. Golden delicious apples as related to their phenolic composition. Journal Of Agricultural And Food Chemistry 52: 4684–4689.
- Committee on Medical Aspects (COMA). 1984. Food Policy Diet and cardiovasculardisease. HMSO. London.

- Dastmalchi, K., Dorman, H.J., Kosarm, D. and Hiltunen R. 2007. Chemical composition and in vitro antioxidant evaluation of a water soluble Moldavian balm (*Dracocephalum moldavica* L.) extract. Lebensmittel Wissenschaft und- Technologie 40: 239- 48.
- Dimitrious, B. 2006. Sources of natural phenolic antioxidants. Trends In Food Sciences And Technology 17: 505–512.
- Foti, M., Piattelli, M., Baratta, M.T. and Ruberto, G. 1996. Flavonoids, coumarins, and cinnamic acids as antioxidants in a micellar system structure-activity relationship. Journal Of Agricultural And Food Chemistry 44: 497–501.
- Gleve, C., Laurens, A., Hocquemiller, R., Laprevote, O., Serani, L. and Cav, A. 1997. Cohibin A and B, acetogenins from roots of *Annona muricata*. Phytochemistry 44(8): 1541-1545.
- Gyamfi, M.A., Yonamine, M. and Aniya, Y. 1999. Free-Radical Scavenging Action of Medicinal Herbs from Ghana: *Thonningia Sanguinea* on Experimentally-Induced Liver Injuries. General Pharmacology 32(6): 661-667.
- Harbourne. 1983. Phytochemical methods. A guide to modern technique of plants analysis. p. 60-64. London: Cahpman Hall.
- Hathcock, J.N. 1985. Quantitative evaluation of vitamin safety. Pharmacy Times 104-113.
- Helsper, J.P.F.G., Hoogendijk, M., Van Norel, A. and Burger-Meyer, K. 1993. Antinutritional factors in faba beans (*Vicia faba* L.) as affected by breeding toward the absence of condensed tannin. Journal Of Agricultural And Food Chemistry 41: 1058-1061.
- Iheanacho, M.E., Udebuani, A.C. 2009. Nutritional Composition of Some Leafy Vegetables Consumed in Imo State, Nigeria. Journal Of Applied Sciences And Environmental Management 13(3): 35–38.
- Jaramillo, M.C., Arango, M.C. Gonzalez, A.S.M. and Velez, R. I. D. 2000. Cytotoxicoty and antileishmanial activity of *Annona muricata* pericarp. Fitoterapia 71: 183-186.
- Kaur, C. and Kapoor, H.C. 2001. Antioxidants in fruits and vegetables – the millennium's health. International Journal Of Food Science And Technology 36: 703– 725.
- Lim, Y.Y., Lim, T.T. and Tee, J.J. 2007. Antioxidant properties of several tropical fruits. A comparative study. Food Chemistry 103: 1003–1008.
- Louli, V., Ragoussis, N. and Magoulas, K. 2004. Recovery of phenolic antioxidants from wine industry byproducts. Bioresource Technology 92: 201–208.
- Magalhaes, L.M., Segundo, M.A., Reis, S., Lima, J.L.F.C. and Rangel, A.O.S.S. 2006. Automatic method for determination of folin- ciocalteu reducing capacity in food products. Journal Of Agricultural And Food Chemistry 54: 5241–5246.
- Mates, J.M. and Sanchez-Jimenez, F.M. 2000. Role of reactive oxygen species in apoptosis: Implications for cancer therapy. International Journal Of Biochemistry And Cell Biology 32: 157–170.

Mccall, M.R. and Frei, B. 1999. Can antioxidant vitamins

materially reduce oxidative damage in humans?.Free Radical Biology And Medicine 26: 1034–1053.

- Meda, A., Lamien, C.E., Romito, M., Millogo, J. and Nacoulma, O.G. 2005. Determination of the Total Phenolic, Flavon-oid and Proline Contents in Burkina Faso Honey, as Well as Their Radical Scavenging Activity. Food Chemistry 91(3): 571-577.
- Minotti, G. and Aust, S.D. 1987. An Investigation into the Mechanism of Citrate- Fe<sup>2+</sup>-Dependent Lipid Peroxidation. Free Radical Biology And Medicine 3(6): 379-387.
- Nieman, D.C., Butterworth, D.E. and Nieman, C.N. 1992. Nutrition. Wm. C. Brown Publishers.Dubuque.
- Ohkawa, H., Ohishi, N. and Yagi, K. 1979. Assay for Lipid Per- oxides in Animal Tissues by Thiobarbituric Acid Reaction. Analytical Biochemistry 95(2): 351-358.
- Onimawo, I.A. 2002. Proximate composition and selected physicochemical properties of the seed, pulp and oil of Soursop (*Annona muricata*). Plant Food For Human Nutrition 57: 165-171.
- Onyechi, A.U., Ibeanu, V.N., Eme, P.E. and Kelechi, M. 2012. Nutrient, Phytochemical Composition and Sensory Evaluation Of Soursop (Annona muricata) Pulp and Drink in South Eastern Nigeria. International Journal Of Basic And Applied Sciences 12(6): 53-57.
- Oyaizu, M. 1986. Studies on Products of Browning Reaction: Antioxidative Activity of Products of Browning Reaction Prepared from Glucosamine. Japanese Journal Of Nutrition 44(6): 307-315.
- Ozioma, A.E., Onisogen, S.E. and Ebirien, P.F. 2013. Proximate and Mineral Composition of Some Nigerian Fruits. British Journal Of Applied Science And Technology 3(4): 1447-1454.
- Pearson, D. 1976. Chemical Analysis of Foods. 7th edn. Churchill. London.
- Puntel, P.R.L., Nogueira, C.W. and Rocha, J.B.T. 2005. Krebs Cycle Intermediates Modulate Thiobarbituric Reactive Species (TBARS) Production in Rat Brain in Vitro. Neurochemical Research 30(2): 225-235.
- Silva, R.M., Andrade, P.B., Valento, P., Ferreres, F., Seabra, R.M. and Ferreira, M.A. 2004. Quince (*Cydonia oblonga* miller) fruit (pulp, peel and seed) and jam: Antioxidant Activity. Journal Of Agricultural And Food Chemistry 52: 4705 – 4712.
- Singleton, V.L., Orthofer, R. and Lamuela-Raven-Tos, R.M. 1999. Analysis of Total Phenols and Other Oxidation Substrates and Antioxidants by Means of Folin-Ciocalteu Reagent. Academic Press, San Diego.
- Spencer, J.P.E., Jenner, A. and Aruoma, O.I. 1994. Intense oxidative DNA damage promoted by L-DOPA and its metabolites: implications for neurodegenerative disease. FEBS Letters 353 (3): 246-444.
- Steel, R.G.D. and Torrie, J.H. 1960. Principles and Procedures of Statistics. McGraw-Hill. London.
- Walker, R.B. and Everette, J.O. 2009. Comparative reaction rates of various antioxidants with ABTS 430 radical cation. Journal Of Agricultural And Food Chemistry 57(4): 1156-1161.
- Zar, J.H. 1984. Biostatistical Analysis Prentice-Hall, Inc., p. 620. Upper Saddle River, NJ.