PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY AND MINERAL COMPOSITION OF SOURSOP (*Annona muricata*) PULP, PEEL AND SEED

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Abstract: Traditionally the outermost parts of most fruits are peeled off and discarded as waste which can constitute environmental pollution. To reduce this, the society must be made to see the value of these parts that are traditionally discarded. This is the basis of this study aimed at investigating the preliminary phytochemical screening, antioxidant properties and mineral composition of the ethanol extract of *Annona muricata* pulp, peel and seed using standard procedures. The results showed the presence of tannins, phenols, steroids, glycosides, terpenoids, alkaloids, flavonoids, resins, carbohydrates and phlobatanins in the pulp. In the peel, tannins, steroids, glycosides, phenols, alkaloids, flavonoids, resins, carbohydrates, phlobatanins and balsams were present while steroids, saponins, terpenoids, flavonoids, volatile oils, carbohydrates, phlobatannins and balsams were present in the seed. At highest concentration of 2 g/ml the pulp had 80.049±0.004% free radical scavenging ability; the peel had 86.797±0.001% while the seed had 77.586±0.002% while ascorbic acid had 54.433±0.001%. The result showed Fe concentration of 0.0138±0.0002 g/ml in the pulp, 0.1449±0.0001 g/ml in the peel and 0.0170±0.0002 g/ml in the seed. Mg concentration was 0.4999±0.0008 g/ml in the pulp, 0.8069±0.0009 g/ml in the peel and 0.7155±0.0002 g/ml in the seed, Cu was 1.0050±0.0002 g/ml in the pulp, 0.0044± 0.0009 g/ml in the peel and 0.0078± 0.0009 g/ml in the seed while Ca was 0.0309±0.0001 g/ml in the pulp, 0.6959±0.0002 g/ml in the peel and 0.3427±0.0016 g/ml in the seed. These results showed that the peel, pulp and seed were rich in phytochemical, mineral nutrients and antioxidant properties, hence the need to encourage their utilisation for pharmacological and nutritional uses.

Keywords: *Annona muricata*, antioxidant, mineral, phytochemical, preliminary screening,

Introduction

Fruits have been important parts of human diet all over the world for ages. Traditionally the outermost parts of most of these fruits are not eaten or used. These unwanted parts are peeled off and discarded as waste. It is expected that as science increasingly supports the need for people to consume more fruits, and national health agencies and international organizations are working together to boost fruit and vegetable consumption around the globe (FAO, 2003), more fruits will be produced and consumed. This expected increase in the consumption of these fruits means more unwanted parts to be discarded. This practice could result to environmental pollution if not well handled. To reduce or avoid this common practice of discarding parts of fruits especially the peels as waste. The society must be made to see the nutritional, medicinal or economic value of the parts that are traditionally discarded. Apart from being a sources of food plants parts have been the basis of traditional medicine system for thousands of years and continue to provide mankind with new remedies and with more research more uses and values of plants can be discovered. With more discovery and awareness of the economic values of plants especially the discarded parts such as fruit peels, the environmental problem due to fruit wastes can be drastically reduced. *A. muricata* is native to the warmest tropical areas in South and North America and is now widely distributed throughout tropical and subtropical parts of the world, including India, Malaysia and Nigeria (Adewole *et al.*, 2006). The fruit is sour and acidic when ripe and grows on an upright and evergreen shrubby tree. It is commonly found in the tropical regions of Western Africa, Central and South America and Southeast Asia (Pinto *et al*., 2005).

All portions of *A. muricata* tree, similar to other *Annona* species, including *A. squamosa* and *A. reticulata* are extensively used as traditional medicines against an array of human ailments and diseases, especially cancer and parasitic infections. The fruit is used as natural medicine for arthritic pain, neuralgia, arthritis, diarrhoea, dysentery, fever, malaria, parasites, rheumatism, skin rashes and worms, and it is also eaten to elevate a mother's milk after childbirth (Mishra, *et al.*, 2013; Adewole *et al.*, 2006; Vieira*et al.*, 2010). In most developing countries, Nigeria inclusive are suffering from epidemic and endemic diseases which lead to daily increase in mortality rate as a result of inadequate intake of balance minerals which are inorganic nutrients, usually required in small amounts from less than 1 to 2500 mg per day, depending on the mineral, Phytochemical and antioxidants are required in the body (Soetan *et al*., 2010). Recently, antioxidants have attracted considerable attention in relation to radicals and oxidative stress, cancer prophylaxis and therapy, and longevity (Kalcher *et al*., 2009). Phenols and polyphenols are the target analyses in many such cases; they may be detected by enzymes like tyrosinase or other phenol oxidases, or even by plant tissues containing these enzymes (Romani *et al*, 2000).

It was predicted that food containing adequate antioxidants, minerals and phytochemicals are relatively expensive and to meet the demand in the developing countries like Nigeria is not easy also the high modern cost of treatment of such deficiency such as accumulation of free radicals in human resulting in diseases such as cancer, fever, etc. It is to this end that intensive efforts are being made to find alternative source of these nutrients from other cheaper sources and effective utilization. A good example of this is soursop (*Annona muricata*) which the peel, pulp and seed are used as ethnomedicinal against tumours and cancer (Adewole *et al.*, 2009). In addition, the fruit is used in the treatment of anti-inflammatory, hypoglycaemic, sedative, smooth muscle relaxant, hypertensive and antispasmodic (Mishra *et al.*, 2013; Adewole *et al.*, 2006). In addition to ethno medicinal uses, the fruits are widely employed for the preparation of beverages, candy, ice creams, shakes and syrups (Jaramillo-Flores *et al*., 2000; Wu *et al*., 1995). This forms the basis of investigating *Annona muricata* fruit (commonly called soursop) for its phytochemicals, antioxidant activities and mineral composition.

Materials and Methods

Collection of samples

The fruits of *A. muricata* were bought from a farmer in Ado market of Karu Local Government Area Nasarawa State, Nigeria. The fruits were identified (Fig. 1) and authenticated in the Department of Plant Science and Biotechnology, Nasarawa State University, Keffi, Nigeria.

Fig. 1: *A. muricata* fruit tree

Fig. 2: *A. muricata* pulp

Fig. 3: *A. muricata* seeds

Preparation and treatment of samples

The peel of the fruit was separated from the pulp and washed to ensure there was no trace of the pulp on the peel. The pulp of the fruit (Fig. 2) was reduced into smaller sizes using a sharp knife for quick and accurate drying. The washed peel, reduced pulp and seed (Figs. 2 and 3) were then air-dried under a shade for 91 h and oven dried at 50^{o}C for 1 h to reduce the amount of moisture present in the samples in order to prevent incomplete extraction of the active ingredients with the choice solvent. The dry samples were separately size-reduced and ground into powder by the use of a mechanical blender and kept in aplastic container for further analysis.

Extraction of A. muricata pulp, peel and seed

The dried pulp, peel and seed (50 g, respectively) were separately weighed and each was transferred into a clean thimble fixed to the Soxhlet extractor. 300 cm^3 of methanol was poured into the round bottom flask with small quantity of boiling chips (to prevent bumping), connected to the Soxhlet apparatus and was heated on a heating mantle at a controlled temperature range (40 - 65^{o}C). The system was allowed to run for about 6-8 h until the coloured sample became colourless. The extract of the sample was filtered and concentrated using a rotary evaporator to dry into powder (Nguta *et al.*, 2011). The resulting dry powder was kept in the fume cupboard for 48 h prior to analyses for phytochemical screening and antioxidant determination.

Phytochemical screening

Chemical test was carried out on the extract for the qualitative determination of phytochemical constituents as described by Harborne (1973); Trease and Evans (1989); Sofowora (1993); Debela (2002).

Test for tannins

About 0.5 g of extract was stirred with about 10 ml of distilled water and then filtered. Few drops of 1% ferric chloride solution were added to 2 ml of the filtrate. The occurrence of a blue –black, green or blue green precipitate indicates the presence of tannins or a dirty green ppt. on addition of few drops of 5% $FeCl_3$ to each of the test extracts.

Test for steroids and triterpenoids

Salkowski test: Crude extract was mixed with chloroform and a few drops of concentrated H_2SO_4 was added. It was shaken well and allowed to stand for some time. Red colour appeared at the lower layer indicated the presence of steroids and formation of yellow coloured layer indicated the presence of triterpenoids.

Test for glycosides

Borntrager's test: 5 ml H_2SO_4 was added to each of the test extracts in a separate test tube. The mixture was heated in boiling water for 15 min. Fehling's solution A and B was then added and the resulting mixture was heated to boiling. A brick red precipitation is obtained.

Test for saponins

To 1g of extract was boiled with 5 ml of distilled water and filtered. To the filtrate, about 3 ml of distilled water was further added and shaken vigorously for about 5 min. frothing from the mixture which persisted even on warming was taken as the presence of saponins.

Test for phenols

0.5 g of the extract was added to 1% ferric (III) chloride in methanol/water (1:1). A dirty green precipitate indicates the presence of phenols.

Test for alkaloids

About 0.5 g of extract was stirred with 5 ml of 1% aqueous HCl on water bath and then filtered. 1 ml of the filtrate was taken individually into 3 test tubes. To 1 ml, Mayers, Wagner and Drangedoffs were added. The formation of precipitate indicates the presence of alkaloids. Mayer's gives a white precipitate or test extracts was acidified with 1% HCl and was then treated with few drops of Mayer, Wagner and Dragendroff's reagents separately in different test tubes. A creamy white (Mayer), reddish brown (Wagner) or an orange brown (Dragendroff's) precipitate indicates presence of alkaloids.

Test for terpenoids

To 0.5 ml of acetic anhydride was mixed with 1 ml of sample extract and a few drops of concentrated H_2SO_4.A bluish green precipitate indicates the presence of terpenoids.

Test for carbohydrate

Fehling's test for reducing sugar: 5 ml of mixture of equal volume of Fehling's solution A and B was added to 2 ml of test extract in a test tube. The resultant mixture was boiled for 2 min. A brick red precipitate of copper (I) oxide indicates positive test.

Test for flavonoids

A small quantity of each test extract was dissolved separately in dilute NaOH. A yellow solution that turns colourless on

addition of concentrated HCl indicates the presence of flavonoids.

Test for phlobatannins

A few drops of 1% HCl was added to 1 ml of extract and boiled. A reddish precipitation indicates the presence of phlobatannins.

Test for resins

About 2 ml of extract was added with equal volume of acetic anhydride solution; then drops of concentrated H_2SO_4 gave colophony resins (violet colouration indicates the presence of resins).

Test for balsams

Three drops of alcoholic $FeCl_3$ were added to 4 ml of extract which was then warmed. A dark green colouration was formed.

Test for volatile oils

A small quantity of the sample was shaken with dil. NaOH and 0.1 M HCl. A white precipitate was formed with volatile oils.

Analysis of antioxidant potential of the fruit extracts

Determination of 2,2-diphenyl-2-picryl hydrazyl(DPPH) radical scavenging capacity

Briefly, 2 ml of various concentrations (0.2 - 1.0 mg/ml) of fruit extract and nutraceutical extracts ranging from 0.2-1.0 mg/ml were added separately to 2 ml of 0.1 mmol/L methanolic solution of DPPH. Incubated for 30 min in the dark room temperature, the absorbance was read against a control using equation:

1% = [(A control – A sample)/A control] X 100

Where $A_{control}$ is the absorbance of the reaction (containing all reagents except the test compound) and Asample is the absorbance of the test compound. The procedures were carried out in triplicates.

Blank absorbance = 0.2712; Note all absorbance are mean values of triplicate readings.

Analysis of mineral elements

Digestion of sample

About 1 g of the dried powdered sample was weighed into a beaker, followed by 10 ml nitric acid (HNO_3) and placed on the hot plate in the fume cupboard to digest. It was allowed to digest until a clean solution was obtained which appear to be light yellow in colouration, after which it was filtered into a 50 ml volumetric flask and was made up to mark with distilled water and analysed using AAS. A blank solution was also prepared. The concentration of mineral element in the sample was calculated as;

Conc. in $g/ml = (conc. - blank \times dilution\ factor) / (wieght\ of\ sample \times 1000))$

Dilution factor = 50 ml

Results and Discussion

Results of the phytochemical screening showed the presence of tannins, phenols, steroids, glycosides, terpenoids, alkaloids, flavonoids, resins, carbohydrates and phlobatanins in the pulp. In the peel, tannins, steroids, glycosides, phenols, alkaloids, flavonoids, resins, carbohydrates, phlobatanins and balsams were present while steroids, saponins, terpenoids, flavonoids, volatile oils, carbohydrates, phlobatannins and balsams were present in the seed (Table 1). The results revealed that steroids, alkaloids, flavonoids, carbohydrate and phlobatannins were positive in all the extract of the soursop (*Annona muricata*). Terpenoids showed negative on the extract of peel and seed while for the pulp it was positive. Triterpenoids was negative in all the extract and tannins, glycosides, phenols and resins were on the peel and pulp extracted. Saponins and volatile oils were negative in on extract of peel and pulp. To some extent the results were consistent with that of Usunobun *et al.* (2014) who reported on the presence of saponins, flavonoids, tannins, alkaloids, carbohydrates, cardiac glycosides and triterpenoids on the leaf extract. It has been reported that alkaloids can be used in the management of cold, fever and chronic catarrh. Alkaloids have wonderful physiological effect on human and used for the development of powerful analgesics drugs (Kam and Liew, 2002; Aremu *et al*., 2016). Interestingly, the most common biological properties of alkaloids are their toxicity against cells of foreign organisms which makes them good antimicrobial agents. Saponin is used as hypercholesterolemia, hyperglycaemia, antioxidant, anticancer, anti-inflammatory and weight loss. It has been reported to have antifungal properties (Tijjani *et al*., 2012). Saponins are expectant, cough depressant and administered for haemolytic activities (Mensah *et al*., 2013). Carbohydrates have many uses in pharmacy. Carbohydrates can be conveniently classified into three main groups: The mono and oligosaccharides (true sugar); the polysaccharides (non-sugar); and the derived carbohydrates (gums, mucilage and pectin). Glycosides are the substance which on hydrolysis yields one or more sugars along with a non- sugar compound. The sugar component is known as the glycone and the non-sugar component is called the aglycone or genin.

Table 1: Preliminary qualitative phytochemical screening of crude extract of *A. muricata* pulp, seed and peel

Phytochemical	Pulp	Peel	Seed
Tannins	+	+	-
Steroids/triterpenoids	+/-	+/-	+/-
Glycosides	+	+	-
Saponins	-	-	+
Phenols	+	+	-
Terpenoids	+	-	-
Alkaloids	+	+	+
Flavonoids	+	+	+
Resins	+	+	-
Volatile oil	-	-	+
Carbohydrate	+	+	+
Phlobatannins	+	+	+
Balsams	-	+	+

+ = Present, - =Absent

Triterpenoids (C_{30}) include steroids, sterols and cardiac glycosides with anti-inflammatory, sedative, insecticidal or cytotoxic activity. Common triterpenes are amyrins, ursolic acid and oleanic acid sesquiterpene like monoterpenes (Martinez *et al*., 2008). The presence of flavonoids may be responsible for the various colours and combinations of colours exhibited by bark, leaves, flowers, fruits and seeds of plants. Flavonoids are known for their antioxidant activity and hence they help to protect the body against cancer and other degenerative diseases (Mensah *et al*., 2013). The presence of tannins in extract pulp and peel of soursop showed that the plants can be used as purgative. They are also used in the treatment of cough, asthma and hay fever (Gills, 1992). Tannins are known to exhibit antiviral, antibacterial and antitumor activities. It was also reported that certain tannins are able to inhibit HIV replication selectivity and also used as diuretic (Tijjani *et al*., 2012). Tannins are known to interact with protein to give the astringent effects which is important for the treatment of ulcer (Adegboye *et al*., 2008, Aremu *et al*., 2017)). Tannins have been found to form irreversible complex with proline-rich protein (Shimada, 2006) resulting in the inhibition of cell protein synthesis. Tannins are well known for their antioxidant and antimicrobial properties as well as for soothing relief, skin regeneration, as anti-inflammatory and dieresis. Phenols also found present in plant sources are major group of compounds acting as primary antioxidant or free radical scavenger (Adesuyi *et al*., 2011).

Results from the present investigation showed that all part of the *A. muricata* fruit are very rich in phytochemicals, even though the phytochemical screening of the three parts revealed a little difference in their constituents. Thus, the preliminary screening tests may be useful in the detection of the bioactive principles and subsequently may lead to exploring novel compounds. Furthermore, these preliminary investigations facilitate the qualitative separation of pharmacologically active chemical compounds. *A. muricata* has been used extensively in folkloric remedies of many diseases, such as malaria, parasites, head lice, and worms (Gajalakshmi *et al*., 2012).

Results obtained for antioxidant properties showed that at highest concentration of 2 g/ml, the pulp had 80.05 ± 0.004% mean free radical scavenging ability, the peel had 88.80±0.001% mean free radical scavenging ability while the seed had 54.43±0.001% free radical scavenging ability. The trend showed a concentration dependent antioxidant activity as this was observed to decrease steadily with decreased sample concentration (Table 2). The DPPH assay provided an easy and rapid way to determine the antioxidant activity of the sample tested in this study. It was found that the peel of *A. muricata* has the highest antioxidant activity followed by the pulp and then the seed. The antioxidant activity reduced drastically as the concentration of the DPPH increased with reduction in the sample concentration. At (2.00 mg/ml) without DPPH which was the first of the percentage antioxidant level where for the peel the value was 86.797±0.001%, pulp (80.049±0.004%) and for the seed (77.58±0.004%). When the concentration of the sample was low at (0.25 and 0.13 mg/ml) the antioxidant value was negative. This means there was no antioxidant activity which implies enough quantity must be consumed to obtain the daily recommended value. From the results, it was found that the antioxidant value of the peel, pulp and seed of *A. muricata* were apparently high in antioxidant value compared to the standard (ascorbic acid) with the exception of the last two concentrations of the seed that gave negative results.

Table 2: Antioxidant activities of *A. muricata* pulp, peel and seed

Conc. (mg/ml)	Percentage DPPH Inhibitions			
	Pulp (%)	Peel (%)	Seed (%)	Ascorbic acid (%)
2.00	80.049±0.004	86.797±0.001	77.586±0.002	54.433±0.001
1.00	79.557±0.003	83.129±0.001	75.862±0.002	52.094±0.005
0.50	78.818±0.009	82.641±0.002	72.167±0.007	48.768±0.002
0.25	75.061±0.003	82.266±0.003	-10.837±0.003	48.645±0.001
0.13	29.310±0.001	33.251±0.024	-12.562±0.002	29.064±0.003

Values are presented as mean ± standard deviation of two determinations

Table 3: Concentration of mineral elements in the pulp, peel and seed of *A. muricata*

Mineral	Pulp(g/ml)	Peel(g/ml)	Seed(g/ml)
Iron (Fe)	0.0138±0.0002	0.1449±0.0001	0.0170±0.0002
Magnesium (Mg)	0.4999±0.0008	0.8069±0.0009	0.7155±0.0002
Copper (Cu)	1.0050±0.0002	0.0044±0.0009	0.0078±0.0009
Calcium (Ca)	0.0309±0.0001	0.6959±0.0002	0.3427±0.0016

Values are presented as mean ± standard deviation of two determinations

Table 3 shows the mineral concentrations of digested sample of the pulp, peel and seeds of *A. muricata* using AAS in mg/L. The peel had the highest concentration of iron, calcium and magnesium compared to the seed and pulp. While in copper concentration was the least. 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 Mg, Cu, Fe, and Ca. Copper is an essential micro- nutrient necessary for the hematologic and neurologic system (Tan *et al*., 2006). Copper is present in all body tissues and plays a role in the formation of connective tissue, and in the normal function of muscles and the immune and nervous systems. Iron is a mineral that is naturally present in many foods, added to some food products, and available as a dietary supplement, iron is an essential component of haemoglobin, an erythrocyte protein that transfers oxygen from the lungs to the tissues. Magnesium has been found to regulate and improve blood sugar control. Calcium is the most abundant mineral in the human body. Calcium is important for optimal bone health, heart, muscles and nerves also need calcium to function properly. Health problems related to weak bones and muscles is as a result of insufficient calcium intake (Audu and Aremu, 2011). The result from this analysis showed that the level of Fe, Mg and Ca were apparently high in the peel and seed of *A. muricata* compared to the pulp of the fruit (Table 3). The peel was apparently high in Mg (0.086±0.0009 g/ml) and calcium (0.6959 ± 0.0002 g/ml) while the pulp was high in Cu with (1.0050±0.0002 g/ml) and the seed was high in Mg, Fe and Ca compared to the pulp. All the results obtained were approximately in line with Akomolafe and Ajayi (2015) who reported on the peel and seed of *A. muricata* with the following results: Mg (0.17±0.07 mg/g) for peel, (0.05±0.07 mg/g) for pulp; Ca (0.60±0.14 mg/g) for peel and (0.61± 0.07 mg/g) for the pulp. The analytical value differences in his results could be trace to location, soil and methodology employed.

Conclusion

From the present result, phytochemical screening for the three extracts showed that the presence of bioactive- metabolites such as alkaloids, flavonoids, carbohydrates, steroids and phlobatannins were found to be present in all the extracts of *A. muricata*. They are known to show medicinal activity as well as exhibiting physiological activity. The peel of *A. muricata* was rich in iron, and can help to balance the iron lost from urine, faeces, gastrointestinal tract, and skin. This study indicates that *A. muricata* peel exhibited significantly high mineral content, antioxidant and phytochemicals constituent compared to the pulp and seed and thus should be explored as a viable source of natural antioxidants for the functional food and pharmaceutical applications. The society should be educated on the nutritional, medicinal and economic values of the peel of *A. muricata.* An increase in the awareness of the usefulness of the seed and peel of the fruit can result in an increase in the demand for the peel which in turn will go a long way of reducing the quantity of environmental waste from the fruit.

Conflict of Interest

Authors declare that there is no conflict of interest related to this study.

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