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Comparative Study on Chemical Composition and Antioxidant Activity of *Annona Muricata* Plant Parts Cultivated in Covenant University, Ota, Ogun State, Nigeria

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Abstract

Annona muricata plant parts possess a broad range of medicinal and biological properties. This research compared the chemical composition and antioxidant properties of *Annona muricata* parts. Proximate, mineral, total phenol and total flavonoid content as well as in vitro antioxidant activity were examined. Results revealed the leaves contained significantly ($p<0.05$) higher composition of moisture (8.69±0.22 %), ash (4.60±0.02 %), protein (14.53±0.11 %), crude fat (10.28±0.03 %), chromium (0.38±0.05 mg/100g), nickel (1.75±0.04 mg/100g), total phenol (1.01±0.03 mg pyrocatechol/mL) and total flavonoid (1.12±0.03 mgGAE/mL) compared to the respective values for root. Carbohydrate (9.29±0.24 %), lead (0.13±0.02 mg/100g) and cobalt (1.93±0.02 mg/100g) composition was significantly lower ($p<0.05$) in the leaves compared to the respective compositions in the root. The leaf and root extract exhibited a concentration-dependent increase in hydroxyl radical scavenging activity with no observable ($p<0.05$) difference in their EC_{50} value. This study suggests the leaves of *A. muricata* found in Covenant University had better chemical composition when compared to the root. Nonetheless, these plant parts may be further exploited for not only their nutritive composition and mineral content but also a natural source of antioxidant agents.

Article History

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Antioxidant,
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Introduction

Plants are a rich bio resource of natural products and phytoconstituents which make them potent as functional foods and for treatment and management of diseases.[1] Despite the availability of various varieties of synthetic drugs which are highly effective

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in curing various diseases, there are people who still prefer using traditional folk medicines and therapy as a result of their less harmful effects. There is a wide diversity of compounds found in plants, especially secondary plant metabolites, identified and isolated from plants.[2] Various studies have shown that these compounds exhibit various properties such as anticancer, antibacterial, analgesic, anti-inflammatory, antitumor and antiviral. Their ethnomedicinal use by the locals, suggests easy access which makes them an easily obtainable source of treatment.[3] *Annona muricata* L. commonly known as soursop, graviola, guanabana and sirsak which belongs to the Annonaceae family, is found in tropical and subtropical regions such as South and Central America, Asia and Africa including Nigeria.[1] Soursop is the most versatile fruit from the Annonaceae family utilised in industrial processes due to its low oxidising rate as well as a huge retrieval of fruit pulp during processing. The fruit is 15-30 cm long with an oval irregular shape and sparse soft green curved spines. It has a white cotton like mesocarp which is fibrous, contains shiny black seeds, a sour taste, pleasing flavour and aroma.[4] Extensive phytochemical evaluations carried out on *Annona muricata* L. different parts (leaves, roots, fruits, stem, bark and seeds) have shown the presence of several bioactive compounds such as alkaloids, megastigmanes, flavonol triglycosides, phenolics, cyclopeptides, essential oils and annonaceous acetogenin compounds. Major minerals such as K, Ca, Na, Cu, Fe and Mg have been reported to be present[5], which makes them ideal dietary sources of electrolytes, essential nutrients and elements which are utilised in various biological processes.[4] The fruit is used to produce juice, candy and sherbets.[6.] The leaves and seeds are known to possess anti-arthritic,[7] anti-cancer,[8] anticonvulsant,[9] antidiabetic and hypolipidemic,[10] anti-inflammatory and anti-nociceptive,[11] antioxidant,[12] antihypertensive[13] and antiparasitic[14] activities. The roots are known for their antihelmintic, antiphlogistic,[14] antiparasitic and pesticidal properties.[6] Despite a broad study on these plant parts, there is a paucity of information on the comparative chemical composition of the plant parts. Hence, the current study was carried out to compare the proximate composition, mineral content and in vitro antioxidant activity of *Annona muricata* leaves and roots found in Covenant University, Ota, Nigeria.

Materials and Methods

Sample Collection and Preparation

The leaves and roots of *Annona muricata* were obtained from Covenant University farm, Canaan land, Ota, Ogun State, Nigeria in December 2013 and identified. Samples were dried for two weeks at room temperature and pulverised. Dried leaf and root powder (50g) was packed into a Soxhlet apparatus and macerated with 250mL methanol at 60–65°C for 3–4h. The extract was filtered through Whatman filter paper No. 1, and the filtrate was concentrated under reduced pressure at 40°C using a rotary evaporator.

Chemicals and Reagents

Sodium Carbonate (Na_2CO_3), Sodium Nitrate ($NaNO_3$), Nitric Acid (HNO_3), Aluminium Chloride ($NaOH$) and Hydrogen Peroxide (H_2O_2), were purchased from Merck, Germany. All other chemicals used were of analytical grade.

Proximate Analysis

Leaves and root of *A. muricata* were pulverised to uniform size and analysed for moisture, crude protein, crude fat, ash, and crude fibre composition according to the standard method of AOAC 15.950.01, 15.976.05, 15.920.39, 15.955.03 and 15.962.09, respectively as described below.[14]

Determination of Moisture Content

Dry crucible in the oven and cool in the desiccator for 15 minutes. Weigh empty petri dish to get (W1), Weigh in 1.5 g (liquid samples) or 2 g (solid samples) into the petri dish and record as (W2) Put in oven for 6-12 hrs at 105 oC. Remove and cool in desiccators. Weigh after cooling to get (W3)

Calculation

% Moisture=(W2-W3)/(W2-W1) × 100

W1 = weight of empty crucible.
W2 = weight of sample and crucible.
W3 = weight of after drying in the oven

Determination of Crude Protein

0.5-1 g of dried samples was taken in digestion flask. 10-15 mL of concentrated H_2SO_4 and 8 g of digestion mixture i.e. K_2SO_4:$CuSO_4$ (8:1) was added to the sample. The flask was swirled in order to mix the contents thoroughly then placed on heater to start

digestion till the mixture became clear (blue green in colour). After 2 hrs the digest was cooled, transferred to 100 mL volumetric flask and the volume was made up to mark by the addition of distilled water. 10 mL of digest was introduced in the distillation tube then 10 mL of 0.5 N NaOH was gradually added. Distillation was continued for at least 10 min and NH_3 produced was collected as NH_4OH in a conical flask containing 20 mL of 4% boric acid solution with few drops of modified methyl red indicator. The distillate was then titrated against standard 0.1 N HCl solution till the appearance of pink colour. A blank was also run through all steps as above.

Calculations

% Crude Protein=6.25x×%N (x=correction factor)

%N=((S-B)×N×0.014×D×100)/(weight of sample×V)

S = Sample titration reading
B = Blank titration reading
N = Normality of HCl
D = Dilution of sample after digestion
V = Volume taken for distillation
0.014 = Milli equivalent weight of Nitrogen.

Determination of Crude Fat

1 g of moisture free sample was wrapped in filter paper, placed in fat free thimble and then introduced in the extraction tube. The receiving beaker was filled with petroleum ether and fitted into the apparatus. After 4-6 siphoning, ether was allowed to evaporate and beaker was removed before last siphoning. Extract was transferred into a clean glass dish with ether washing and evaporated ether on water bath. Dish was placed in an oven at 105°C for 2 hrs and cooled in a desiccator.

Calculation

% Crude fat=(weight of ether sample×100)/(weight of sample)

Determination of Ash

For the determination of ash, clean empty crucible was placed in a muffle furnace at 600°C for an hour, cooled in desiccator and then weight of empty crucible was noted (W1). One gram of each of sample was taken in crucible (W2). Then the crucible was placed in muffle furnace at 550 °C for 2-4 h. The appearances of grey white ash indicated complete oxidation of all organic matter in the sample. After ashing furnace was switched off. The crucible was cooled and weighed (W3).

Calculation

% Ash=(W3-W2)/(W2-W1)×100

W1 = weight of empty crucible.
W2 = weight of sample and crucible.
W3 = weight of after drying in the oven

Determination of Crude Fibre

0.153 g of sample was weighed (W0) and transferred to porous crucible. 150 mL of preheated H_2SO_4 solution and some drops of foam-suppresser was added to each column. Sample was boiled and left for 30 mins. Valves were opened for drainage of acid and rinsed with distilled water thrice to completely ensure the removal of acid from sample. The same procedure was used for alkali digestion by using KOH instead of H_2SO_4. Sample was oven dried at 150°C for 1 hr, then cooled in a desiccator and weighed (W1). The sample crucibles were kept in muffle furnace at 55°C for 3-4hrs and later cooled in a desiccator and weighed again (W2).

Calculations

% Crude fibre=(W1-W2)/W0×100

Determination of Carbohydrate

Carbohydrate was calculated by difference after analysis of all the other items method in the proximate analysis, i.e. 100 - (moisture + crude protein + crude fat + crude fibre + ash).

Mineral Determination

The official method of AOAC[15] was adopted for the mineral analysis of the samples: 1 g of the sample was weighed in a Vycor dish, dried for 1hr at 150°C in air forced oven and then ashed overnight (16h) at 550 °C before cooling in a desiccator. One mL of HNO_3 was added to dissolve the ash. The sample is then transferred to a 250 mL volumetric flask and made up to volume with H_2O. Sodium (Na) and potassium (K) levels of the samples were ascertained using a flame emission photometer

with NaCl and KCl as standards. All other metals were determined by atomic absorption spectrometry (AAS) method.[16]

Total Phenol Quantification

Total phenolic content of the extracts was analysed using the Folin-Ciocalteau reagent method described by Lee *et al.,*[17] with slight modification. 10 µL of the extract was added to 600 mL of distilled water followed by 50 µL of 10% of Folin-Ciocalteau reagent. 150 µL of 7% Na_2CO_3 was added and vortexed. The mixture was left at room temperature for 8 mins before 190 µL of water was added and kept at room temperature for 2 hrs. Absorbance was read at 765 nm. Total phenolic content was calculated from the calibration curve, and the results were expressed as mg of pyrocathecol equivalent per mL.

Total Flavonoid Quantification

The total flavonoid content of the crude extract was determined by the aluminium chloride colourimetric method described by Baba and Malik18, with slight modification. Distilled water (490 µL) was added to 10 µL of extract, 30 µL of 5% Sodium Nitrate, 30 µL of 10 % AlCl and incubated at room temperature for 5 mins. One M NaOH followed by 240 µl of H_2O was added and vortexed thoroughly. Absorbance was read at 510 nm. The total flavonoid content was calculated from a calibration curve, and the result was expressed as mg gallic acid equivalent per mL.

Hydrogen Peroxide Scavenging Activity

The ability of extract to scavenge H_2O_2 was determined according to the method of Sharma *et al.,*[19] with slight modification. Hundred µL of extract was incubated with 0.6 mL of H_2O_2 (40mM in a phosphate buffer, 0.1M pH 7.4) in dark for 10 mins. A negative control was set up in parallel with entire reagent except for either extract or standard. The absorbance of H_2O_2 at 230 nm was determined against a blank solution containing phosphate buffer.

Calculation

Scavenging ability on hydroxyl radicals (%)= $(A_{control}-A_{test} / A_{control})\times 100$

Where

$A_{control}$ is the absorbance of the control reaction

A test is the absorbance in the presence of the sample extract.

Statistical Analysis

Results were expressed as mean ± standard error of mean (SEM) of triplicate values. Statistical analysis was performed by one-way ANOVA followed by Duncan test as post hoc. IBM SPSS statistics 23 was used and a probability (p) value < 0.05 was considered to be statistically significant.

Result

Proximate Composition

The proximate composition as reported in table 1 on a dry weight basis showed there was a significantly ($p<0.05$) higher composition of moisture, ash, crude fat and protein in the leaves (8.69±0.22 %, 4.60±0.02 %, 10.28±0.03 % and 14.53±0.11 %) than that of the roots (2.40±0.03 %, 1.20±0.06 %, 6.46±0.04 % and 7.53±0.11 %). Carbohydrate was significantly ($p<0.05$) higher in the root (27.23± 0.14 %) than the leaves (9.29±0.24 %).However, the crude fibre composition of leaves (52.63± 0.36 %) was not significantly ($p<0.05$) different from that of the roots (55.19±0.02 %).

Mineral Composition

Mineral content as revealed in table 2 revealed chromium and nickel were significantly ($p<0.05$) higher in leaves (0.38±0.05, 1.75±0.04 mg/100g) compared to the root (0.30±0.05, 1.25±0.03 mg/100g) respectively. Lead and cobalt content was significantly ($p<0.05$) higher in roots

Table 1: Proximate analysis of *A. muricata* leaves and roots

SAMPLE	LEAF	ROOT
Moisture (%)	8.69 ± 0.22^a	2.40 ± 0.03^b
Ash (%)	4.60 ± 0.02^a	1.20 ± 0.06^b
Crude Fats (%)	10.28 ± 0.03^a	6.46 ± 0.04^b
Crude Fibre (%)	52.63 ± 0.36^a	55.19 ± 0.22^a
Protein (%)	14.53 ± 0.11^a	7.53 ± 0.11^b
Carbohydrate (%)	9.29 ± 0.24^a	27.23 ± 0.14^b

Values are expressed as mean ± SEM of 3 replicates.
[ab]Values with different superscript on a row are significantly different ($p<0.05$)

(0.23±0.05, 2.80±0.04 mg/100g) compared to the leaves (0.13±0.02, 1.93±0.02 mg/100g) respectively. There was no significant ($p<0.05$) difference between the amount of zinc, cadmium, copper, magnesium, sodium, calcium, potassium and iron detected in the leaves and root.

Total Phenolic and Flavonoid Quantification

Results recorded in table 3 show total phenolic and flavonoid content was significantly ($p<0.05$) higher in the leaves (1.01±0.03 mg pyrocatechol/mL, 1.12±0.03 mg GAE/mL) compared with the root (0.74±0.02 mg pyrocatechol/mL, 0.25±0.01 mg GAE/mL). There was no significant ($p<0.05$) difference in the EC50 value for hydroxyl radical scavenging activity of the leaf (2.92±0.02) and root extract (2.65±0.03).

Hydroxyl Radical Scavenging Activity

A concentration-dependent increase in hydroxyl radical scavenging activity of *A. muricata* leaf and root methanolic extract was depicted in figure 1. There was, however, a slight scavenging activity decrease in the highest concentration of the leaf extract.

Discussion

The results from the proximate composition of the leaves and root of *A. muricata* further show their nutritive and medicinal properties. Cellular

Table 2: Mineral content of *A. muricata* leaves and roots

SAMPLE	LEAF	ROOT
Zinc (mg/100g)	8.70±0.29[a]	8.40±0.18[a]
Cadmium (mg/100g)	5.23±0.09[a]	5.50±0.10[a]
Chromium (mg/100g)	0.38±0.05[a]	0.30±0.05[b]
Lead (mg/100g)	0.13±0.02[a]	0.23±0.05[b]
Copper (mg/100g)	7.35±0.11[a]	7.80±0.09[a]
Nickel (mg/100g)	1.75±0.04[a]	1.25±0.03[b]
Cobalt (mg/100g)	1.93±0.02[a]	2.80±0.04[b]
Magnesium (mg/100g)	30.73±0.21[a]	32.80±0.27[a]
Sodium (mg/100g)	49.88±0.12[a]	51.28±0.20[a]
Calcium (mg/100g)	155.03±0.39[a]	151.30±0.57[a]
Potassium (mg/100g)	23.95±0.19[a]	24.88±0.44[a]
Iron (mg/100g)	37.55±0.23[a]	38.55±0.88[a]

Values are expressed as mean ± SEM of 3 replicates. [ab]Values with different superscript on a row are significantly different ($p<0.05$)

Table 3: Total phenolic, flavonoid composition and EC_{50} values for hydroxyl radical scavenging activity

SAMPLE	Leaves	Roots
Total Phenol (mgpyrocatechol/mL)	1.01±0.03[a]	0.74±0.02[b]
Total Flavonoid (mgGAE/mL)	1.12±0.03[a]	0.25±0.01[b]
EC_{50}	2.92±0.02[a]	2.65±0.03[a]

Values are expressed as mean ± SEM of 3 replicates. [ab]Values with different superscript on a row are significantly different ($p<0.05$)

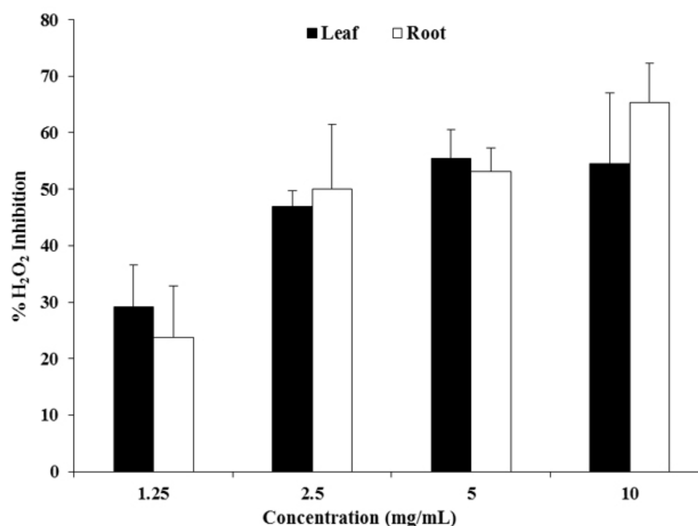

Fig. 1: Hydroxyl radical scavenging activity of methanolic extract of leaves and roots of *A. muricata*. Values are expressed as mean ± SEM of 3 replicates

metabolism thrives on and requires energy provided by carbohydrate to run continuously.[20] Dietary fibre improves motility in the digestive system and plays a role in cardiovascular diseases and cancer prevention.[21] The amount of crude fibre present in the leaf and root of *A. muricata* in this study, were very high compared to that of seed previously reported. However, the inverse was the case for the carbohydrate content as it was higher in the seed.[22] The high carbohydrate and fibre content of the root shows that it can be used to aid digestion and as a source of energy.[23] The moisture content of the leaves suggests it may be a better source of hydration, but nonetheless, it may be more prone to microbial attack during storage than the roots. This indicates that the leaf may have reduced shelf life which corresponds with a study on *Costus afer*[24] as a high moisture content of any biological matter makes it highly susceptible to microbial attack during storage.[25] The high crude fat, protein and ash contents of the leaf suggest that they could be used as a better source of plant fat, protein and minerals in feed supplementation. Fat is an essential macromolecule needed for energy and absorption of fat-soluble vitamins.[26] Proteins are essential components of diets required for energy generation, maintenance of body tissues and synthesis of enzymes, hormones as well as other substances required for healthy functioning.[27,28] The crude protein content of the leaves and roots shows *A. muricata* may be used as a natural protein supplement for animals. The protein and fat content of the leaves are higher than the 12.5% and 1.49% reported for *Nauclea latifolia* leaf but lower than the reported protein (27.74% and 20.72%) content for *Vitex doniana* and *Moringa oleifera* leaves respectively.[29] The reported high fat content of the leaves and root were in line with the reported values (8.3 – 27.0%) of leafy vegetables.[29] It is also corroborated by the findings of Agu and Okolie[30] on the leaf and root extracts which may be as a result of the high presence of annonaceous acetogenins which are long chain fatty acids derivative. Minerals play a massive role in metabolic pathways, disease prevention and management. Their presence in *A. muricata* leaves and roots may be the reason for the folkloric use in treatment and management of diseases.[24] High concentration of minerals was observed to be present in the leaves and root of *A. muricata* with calcium, sodium, iron, magnesium and potassium being the most abundant. Calcium is essential for healthy bones, teeth, blood, muscles[31], as well as absorption of dietary vitamin B, for the synthesis of the neurotransmitter acetylcholine and immune response.[32] Magnesium, potassium as well as calcium are involved in enzyme synthesis, cofactors for enzyme activation, biological structure promoter and optimal physiological function.[33,34]

Sodium is required for optimal acid-base balance, maintenance of osmotic pressure and cellular homeostasis.[35] Iron is important for animal survival as it plays a role in respiration, DNA synthesis and blood functioning.[36] Chromium prevents diabetes by being directly involved in insulin production and function.[37] All other minerals present in the leaves and root play a part in the optimal function of various physiological and biochemical processes.[38] Interestingly results of mineral content analysis of the leaves were in contrast with that of Usunomena and Paulinus[39] except chromium which had almost similar value. This observation, may be as a result of the soil location, where this plant was cultivated, as well as the environmental practices of the surrounding populace. Phenolic substances are abundant low molecular weight bioactive compounds in plants which have various health benefits.[40] The dose-dependent increase in the scavenging capacity of the leaves and roots may be attributed to the presence of flavonoids and phenolics in the methanolic extracts, as they are known to be soluble in polar solvents.[24] These plant secondary metabolites are known to possess potent antioxidant capacity which may be as a result of the presence of the hydroxyl groups present in their ring structures. These groups are effective in scavenging of reactive oxygen species by donating and accepting electrons with free radicals thereby quenching them.[36,41] The findings agree with the studies carried out by Agu and Okolie[30], Kalidindi *et al.,*[42] and Mariod *et al.,*[43] attributing the antioxidant properties of their plants to the phenol and flavonoid content.

Conclusion

This study suggests the leaves of *A. muricata* have more phenolic and flavonoid contents compared to the root. Nonetheless, these plant parts are a rich source of nutrients and can be capitalised for feed supplementation, the potential to supply sufficient amount of minerals for consumers and microbial media for microorganisms. Besides their nutritional and mineral value, they possess antioxidant activity, thus may be utilised as an alternative natural source of antioxidants.

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Conflicts of interest

No conflict of Interest.

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