Research Article

Comparative evaluation of proximate composition and anti-sickling potential of *Annona muricata* Linn seeds and leaves

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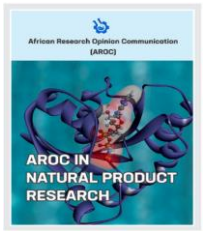

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Abstract

Background: *Annona muricata* Linn is a notable, well-studied plant of therapeutic value. Based on the abundant pharmacological constituents contained in the understudied plant, it is imperative that the plant parts are investigated for nutritional value and anti-sickling potentials. Sickle cell anaemia (SCA) is an inheritable haematological disorder, caused by an amino acid substitution on the haem protein. The outcomes for SCA are poor health indices and high mortality. Therefore, the use of natural products is necessary and widely promoted in countries with poor health infrastructure. **Methods:** In this study, *A. muricata* seeds and leaves were comparatively analysed for the proximate compositions. In addition, aqueous and ethanol extracts of *A. muricata* seeds and leaves were respectively analysed for anti-sickling potentials with the use of spectrophotometry. **Results:** Proximate composition of *A. muricata* seeds and leaves showed that both plant parts contain ash, carbohydrate, fat, fibre, moisture and protein. However, percentage proximate composition of *A. muricata* seeds was not significantly different from the percentage proximate composition of *A. muricata* leaves ($p \leq 0.05$). From anti-sickling analysis, the aqueous extracts of *A. muricata* seeds and leaves were observed to inhibit HbSS polymerisation, while the ethanol extracts of *A. muricata* seeds and leaves showed limitations to the inhibition of HbSS polymerisation. **Conclusion:** *A. muricata* seeds and leaves possess potentials as health or nutritional supplements for the management of SCA. Further studies are necessary in order to ascertain efficacy and safety in *in vivo* models.

Keyword: *Sickle cell anaemia; proximate composition; anti-sickling; Annona muricata Linn; medicinal plants*

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1.0 Introduction

Plants are the major sources of medicinal compounds. Plant-derived medicinal compounds have been extracted and used as therapeutic interventions for a wide range of diseases and maladies [1-2]. Medicinal plants are basic and crucial for the preservation of health and medical care worldwide [1,3].

Annona muricata L. belongs to the Annonaceae family. It is known by its English name "sour-sop" in most parts of Nigeria and is identified as Graviola, guanabana, sirsak in some tropical and subtropical parts of the world. *A. muricata* is a tree of about 8 meters in height that possesses glossy green leaves and thrives in warm tropical regions of the world. The fruits of the *A. muricata* tree are heart-shaped and are distinctly covered in blunt spiky green skin, which gains a yellow colour when ripe [4].

A. muricata has been well-studied and found to be potentially effective as an anti-parasite, an insecticide, an anti-helminthic, an antipyretic and an anti-inflammatory agent. This remarkable plant, *A. muricata* has been reported to be useful in respiratory illnesses, malaria, gastrointestinal disorders, liver diseases, kidney diseases, heart diseases, diabetes, low blood pressure and many cancer types [2,5]. Although all parts of the *A. muricata* plants have been reported to possess some level of therapeutic value in traditional

medicine [2], this study will be concerned with the seeds and leaves of the *A. muricata* plants.

Sickle cell anaemia (SCA), otherwise known as HbSS is a fatal inheritable disease that lives with patients throughout life. The prevalence of SCA is observed uniquely in distinct populations in Africa, South America, North America and the Middle East [6]. The well-known treatment for SCA is the bone marrow transplant from a matching donor. In the absence of a suitable bone marrow donor, life-long disease management is unavoidable. The management of SCA with therapeutic regimens are based on two strategies: the first strategy deals with the reduction of HbSS cellular levels through agents such as hydroxyurea, which alternatively increases the levels of foetal haemoglobin (HbF) or the inhibition of ion transport pathways that facilitate erythrocytic hydration. The second strategy involves the reduction of adhesiveness and aggregation of sickle red blood cells [6-8].

Across the globe, several variants of SCA have been identified, which include HbSS, HbC and sickle cell β-thalassemia. Individuals affiliated with HbAS, otherwise known as the sickle cell heterozygous trait (HbAS) show no clinical manifestations but can produce homozygous HbSS offsprings with a HbAS partner [6, 9]. By the mutational substitution of an amino acid on either the α-globin chain or the β-globin chain of the haemoglobin, a variant occurs. Normal haemoglobin possesses glutamic acid, a hydrophilic amino acid on the sixth amino acid position of its β-globin chain. In the case of HbSS, the amino acid present in the sixth position of the β-globin chain is valine, a hydrophobic amino acid [6]. The amino acid substitution of glutamic acid by valine leads to the formation of abnormal haemoglobin molecules with immense haematological consequences. With HbSS individuals, hypoxic conditions result in deoxygenated HbSS cells (deoxy-HbSS), this causes the red blood cells to become sickle and clumpy, usually termed as HbSS polymerisation [6, 9-10].

The aim of this study is to evaluate and compare the proximate compositions and *in vitro* anti-sickling abilities of the seeds and leaves of the *A. muricata* plant. The information generated from the investigation of these selected plant parts of *A. muricata* should provide additional pharmacological knowledge and complementary nutritional constitution for SCA management.

2.0 Materials and methods

2.1 Plant materials

Fully mature *A. muricata* fruits were harvested and *A. muricata* leaves were collected from trees in Nsukka, Enugu State, Nigeria. The fruits and leaves were identified and authenticated by Dr. Hyginus C. Ogbuehi of the Department of Crop Science and Biotechnology, Imo State University, Owerri, Nigeria. Taxonomic serial number 18098 was assigned after identification.

2.2 Preparation of the plant materials

A. muricata leaves and fruits were washed several times with distilled water to remove dirt. To retrieve the *A. muricata* seeds, several fruits were dissected, retrieved, and washed many times to remove traces of the fruit pulp. The leaves and seeds were dried at room temperature and subsequently grounded to pulverised form.

2.3 Proximate analysis

Proximate composition of the seeds and leaves of *A. muricata* were determined using the methods of A.O.A.C., [11-12].

2.3.1 Moisture content: Petri-dishes were washed, oven-dried and weighed. 2 g of pulverised *A. muricata* seeds and leaves respectively were placed in Petri-dishes. Investigated samples were subsequently placed in an oven and heated for 1 hour at 100°C. At this time, weights were noted. Samples were intermittently dried in the oven at 100°C for 1 hour until steady weights were obtained. The moisture content of investigated samples was obtained by subtracting the final steady sample weight from the initial steady sample weight.

2.3.2 Ash content: Platinum crucibles were washed, dried and weighed. 2 g of pulverised *A. muricata* seeds and leaves respectively were weighed into respective crucibles and placed on a muffle furnace for 3 hours at 500°C. The percentage of Ash content was determined with the formula below.

$$(W_3 - W_1)/(W_2 - W_1)\ x\ 100$$

Where; W_1; weight of empty platinum crucible, W_2; weight of platinum crucible and sample before burning; W_3; weight of platinum and ash.

2.3.3 Crude fiber: A 2 g of pulverised *A. muricata* seeds and leaves were weighed and defatted with petroleum ether. Defatted samples were boiled under reflux with H_2SO_4, filtered and then washed to remove traces of acid and afterwards transferred into a beaker. Carbonated free NaOH was added and filtered, residues were oven-dried, then cooled and weighed. The % crude fiber was calculated using the formula:

100 x (weight of fiber/weight of sample)

2.3.4 Crude fat: Using the Soxhlet fat extraction method, investigated *A. muricata* leaves and seeds were extracted with 300 ml petroleum ether in an already weighed empty boiling flask. Boiling flasks were transferred into an oven, cooled and weighed. The calculated difference between the boiling flask and the boiling flask with extract determines the crude fat content.

2.3.5 Crude proteins: To determine protein content, the Macro Kjeldahl method was utilised. Protocol adopted from A.O.A.C. [11].

2.3.6 Carbohydrate determination: To determine the carbohydrate content, the differential method was used. The % carbohydrate:

100- (%proteins+%moisture+%ash+%fat+%fiber)

2.4 Anti-Sickling analysis

2.4.1 Extract preparations

For the preparation of ethanol extract, 100 g of pulverised seeds and leaves of *A. muricata* respectively were soaked with 1000 ml of 80% ethanol and extracted via maceration. The filtered extract was recovered at 49°C by evaporation, and the semi-solid extract was stored at 4°C before analysis. For the preparation of aqueous extract, 100 g of pulverised seeds and leaves of *A. muricata* were soaked with 1000 ml of distilled water and extracted via maceration. The filtered extract was recovered at 49°C by evaporation, and the semi-solid extract was stored at 4°C before analysis.

2.4.2 Preparation of analyte blood samples:

The blood sample was collected by venipuncture from confirmed HbSS (homozygous) sickle cell disease patients attending the clinic at the Federal Medical Center, Owerri, Nigeria. Permission for the use of blood was granted by the bio-ethics committee of the hospital. A 2 ml blood sample was collected into EDTA bottles and carefully mixed. Erythrocytes were isolated from the blood samples by centrifugation at 2,000 rpm for 10 minutes. The blood plasma was carefully aspirated and the sedimented erythrocytes were suspended and washed twice in normal saline (0.9% NaCl). Erythrocyte suspension was freeze-thawed at 0°C to produce a haemolysate solution for the haemoglobin polymerization experiment.

2.4.3 Haemoglobin polymerization inhibition analysis:

The method of Nwaoguikpe and Uwakwe, [13] was modified by monitoring the turbidity at 555 nm instead of 700 nm [14]. 2 ml of freshly prepared 2% sodium metabisulfite ($Na_2S_2O_5$), 2 ml of normal saline and 0.1 ml of the HbSS haemolysate solution were pipetted into a cuvette, shaken and the polymerization of HbSS was monitored with a digital UV-VIS spectrophotometer for 30 minutes. For test samples and exclusive of normal saline, varying concentrations of the extracts: 100, 200, 300 and 500 µg/ml were utilized respectively.

The rate of polymerisation (ROP) per minute was calculated with the formula below:

$$R_p = (OD_f - OD_i) / t$$

Where: (R_p) = rate of polymerisation, (OD_f) = final absorbance / optical density at time (30 minutes), (OD_i) = initial absorbance / optical density at time zero, and (t) = time of assay in minutes.

To determine the % relative polymerization (%rP), the formula below was used:

$$\%rP = (Rp_t / Rp_c) * 100,$$

where Rp_t = rate of polymerisation of treatment (for each extract concentrations), Rp_c = rate of polymerisation of control (zero treatment).

The relative inhibition (RI) values were derived by the formula below:

$$RI = \%rP_c - \%rP_t ,$$

where $\%rP_c$ = % relative polymerisation of control, $\%rP_t$ = % relative polymerisation of treatment (for each extract concentration).

2.5 Statistical analysis:

Statistical analysis was used to test proximate composition values obtained from seeds against the proximate composition values obtained from leaves. The GraphPad software (CA, USA) and Microsoft Excel (USA) was utilised to conduct statistical analysis. Statistical paired and unpaired T-test were conducted separately. Statistical significance was considered at $p \leq 0.05$.

3.0 Result

3.1 Proximate composition of *A. muricata* Linn seeds and leaves

Based on the proximate composition analysis, the seeds and leaves of *A. muricata* L. were comparatively analysed and represented in Table 1. The ash content, carbohydrate content, fat content, fibre content, moisture content and protein content were investigated respectively. From the results obtained, the proximate composition of the seeds and leaves of *A. muricata* are different. However, statistically, the proximate composition of seeds and leaves are not significantly different ($p \leq 0.05$). From table 1. the seeds of *A. muricata* were shown to have lower ash and carbohydrate content than the leaves of *A. muricata*. In addition, the seeds were observed to possess higher fat, fibre, moisture and protein content than the leaves of *A. muricata* plant.

3.2 Anti-sickling potential of *A. muricata* Linn seeds and leaves

The rate of polymerisation, relative percentage polymerisation and relative percentage inhibition of variable concentration of *A. muricata* Linn seeds and leaves against HbSS is shown in table 2. The aqueous extract of *A. muricata* seeds inhibited haemoglobin polymerisation dose-dependently. The aqueous extract of *A. muricata* leaves was also observed to inhibit haemoglobin polymerisation dose-dependently with a decline at 200 µg/ml (Figure 1). The ethanol extract of *A. muricata* seeds and leaves respectively showed maximum inhibitory effect on sickle cell haemoglobin polymerisation at 100 µg/ml, afterwards a steady decline of polymerisation inhibition was observed.

Table 1: Proximate composition of seeds and leaves of *A. muricata* Linn

	***A. muricata* seeds**	***A. muricata* leaves**
Ash (%)	2.11 ± 0.61	4.03 ± 0.35
Carbohydrate (%)	73.27 ± 0.28	79.55 ± 0.29
Fat (%)	8.14 ± 0.05	7.50 ± 0.18
Fiber (%)	2.49 ± 0.27	2.31 ± 0.10
Moisture (%)	4.42 ± 0.15	1.72 ± 0.11
Protein (%)	9.75 ± 0.07	4.88 ± 0.08

Results are means of triplicates readings ± standard deviations.

4.0 Discussion

In this study, the proximate composition and anti-sickling potential of seeds and leaves of *A. muricata* Linn were evaluated through *in vitro* techniques. Proximate composition of investigated samples: the seeds and the leaves of *A. muricata* were not significantly different ($p \leq 0.05$). Numerous studies have conducted similar investigations on plant parts of *A. muricata*, however none have evaluated the seeds and the leaves comparatively. Proximate composition of *A. muricata* seeds and leaves previously reported [15-16] are agreeable with results obtain from this study. Although differences exist in the proximate composition of investigated *A. muricata* seeds and leaves, these differences are not statistically significant. Both seeds and leaves of *A. muricata* possess basic nutritional value, which is useful in pharmaceutical formulation of dietary supplements in HbSS management.

Due to the demand for an improved anti-sickling regimen in the management of SCA, this study investigates the effect of *A. muricata* seeds and leaves on HbSS polymerisation. Results indicate that there is favourable potential of the understudied plant parts. The aqueous extracts of *A. muricata* seeds and leaves show potential in inhibiting HbSS polymersation and reversing HbSS sickling. Only aqueous extracts of *A. muricata* seeds and leaves were able to inhibit HbSS polymerisation dose-dependently.

Table 2: Rate of polymerisation, relative polymerisation (%) and relative inhibition of variable concentration of *A. muricata* Linn seeds and leaves respectively against HbSS specimen.

Sample	Concentration (µg/ml)	*Rate of polymerisation	%Relative polymerization(%rP)	Relative inhibition (RI)
Seeds (aqueous extract)	0	-0.0023 ± 0.000040	100	0
	100	0.0007 ± 0.000010	-30.44	130.44
	200	0.0016 ± 0.000055	-69.57	169.57
	300	0.0023 ± 0.000126	-100	200
	500	0.0052 ± 0.000351	-226.09	326.09
Seed (ethanol extract)	0	-0.0023 ± 0.000040	100	0
	100	0.0039 ± 0.000055	-169.57	269.57
	200	0.0023 ± 0.000153	-100	200
	300	0.001 ± 0.000321	-43.48	143.48
	500	-0.0015 ± 0.000051	65.22	34.78
Leaves (aqueous extract)	0	-0.0023 ± 0.000040	100	0
	100	0.0017 ± 0.000047	-73.91	173.91
	200	0.0011 ± 0.000020	-47.83	147.83
	300	0.0027 ± 0.000180	-117.39	217.39
	500	0.0055 ± 0.000153	-239.13	339.13
Leaves (ethanol extract)	0	-0.0023 ± 0.000040	100	0
	100	-0.001 ± 0.000153	43.48	56.52
	200	-0.0012 ± 0.000058	52.17	47.83
	300	-0.0012 ± 0.000300	52.17	47.83
	500	-0.0014 ± 0.000153	60.87	39.13

*Rate of polymerisation values are means of triplicate readings ± standard deviation

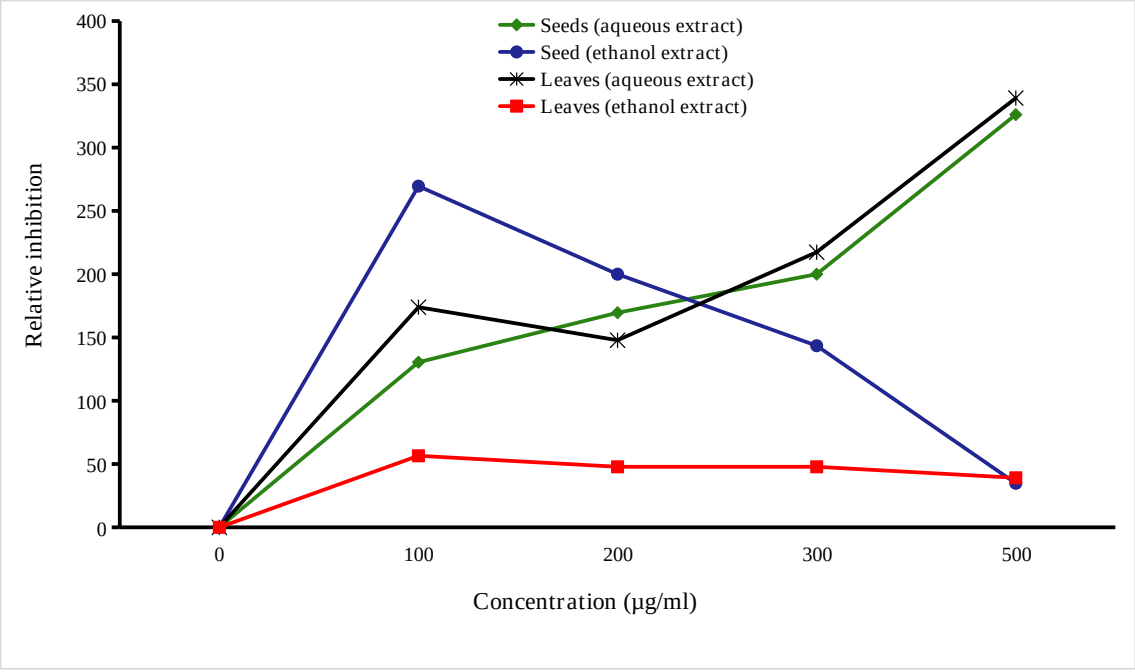

Figure 1: Effect of variable concentrations of aqueous and ethanol extracts of seeds and leaves of *A. muricata* Linn on the inhibition of HbSS polymerisation.

Ethanol extracts of *A. muricata* seeds and leaves were not effective in the inhibition of HbSS polymerisation, further studies are needed to assess the effect of ethanol on the red blood cells or on HbSS polymerisation.

It has been previously reported that identified plant extracts with potentials to inhibit HbSS polymerisation or HbSS sickling reversal usually possess phyto-compounds that target and inhibit the calcium-activated potassium channels [9]. It is understood that increased intracellular calcium, increases the efflux of potassium ions and water, which leads to dehydration. The inhibition of the calcium-activated potassium channels leads to increased intercellular potassium levels, increased hydration and reversal of the HbSS cells sickling [9, 17]. It is suggestive that the inhibition to HbSS polymerisation by the aqueous extracts of *A. muricata* seeds and leaves may have been effective through the inhibition of the calcium-activated potassium channels and the hydration of the red blood cells.

From these preliminary findings, *A. muricata* seeds and leaves show potential in the management of SCA. The seeds and leaves of the understudied plant are nutritive and have the ability to inhibit HbSS polymerisation. Further studies are needed to investigate *A. muricata* leaves and seeds in *in vivo* models and conduct cytotoxicity studies in order to ascertain safety of aqueous extracts of *A. muricata* seeds and leaves in the management of SCA.

5.0 Conclusion

Based on the present study results, it is suggestive that the seeds and leaves of *A. muricata* plants possess nutritive value. Furthermore, the aqueous extracts of the investigated plant parts are potential anti-sickling regimens due to their effect on polymerisation. Further analytical studies will be required to ascertain the pharmacological value of *A. muricata* plant parts.

Conflicts of interest:

All authors have no conflict of interest to declare.

Authors contributions:

CHO and CCN conducted proximate analyses on investigated plant parts, CHO, RTN and CGN conducted anti-sickling assessment of investigated plant samples on HbSS sample. CHO supervised the study. CHO, CSC and FNU prepared and edited the original draft of the manuscript.

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References

1. Ncube NS, Afolayan AJ, Okoh AI. Assessment techniques of antimicrobial properties of natural compounds of plant origin: current methods and future trends. African journal of biotechnology. 2008;7(12).

2. Coria-Téllez AV, Montalvo-Gónzalez E, Yahia EM, Obledo-Vázquez EN. Annona muricata: A comprehensive review on its traditional medicinal uses, phytochemicals, pharmacological activities, mechanisms of action and toxicity. Arabian Journal of chemistry. 2018;11(5):662-91.

3. World Health Organization, Public Health Agency of Canada, Canada. Public Health Agency of Canada. Preventing chronic diseases: a vital investment. World Health Organization; 2005.

4. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, Kadir HA. Annona muricata (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. International journal of molecular sciences. 2015;16(7):15625-58.

5. Gavamukulya Y, Wamunyokoli F, El-Shemy HA. Annona muricata: Is the natural therapy to most disease conditions including cancer growing in our backyard? A systematic review of its research history and future prospects. Asian Pacific journal of tropical medicine. 2017;10(9):835-48.

6. Igwe CU, Alisi CS, Nwaoguikpe RN, Ojiako AO, Onwuliri VA. Relationship between Antisickling Potency of Edible African Plants and their Amino Acid Compositions. Nigerian Journal of Biochemistry and Molecular Biology. 2017;32(2):110-9.

7. De Franceschi L, Corrocher R. Established and experimental treatments for sickle cell disease. haematologica. 2004;89(3):348-56.

8. Agrawal RK, Patel RK, Nainiwal L, Trivedi B. Hydroxyurea in sickle cell disease: drug review.

Indian Journal of Hematology and Blood Transfusion. 2014;30(2):91-6.

9. Okpuzor J, Adebesin O, Ogbunugafor H, Amadi I. The potential of medicinal plants in sickle cell disease control: A review. International Journal of Biomedical and Health Sciences. 2021;4(2).

10. Azubuike JC, Nkanginieme KE. Sickle cell Disease in Paediatrics and Child health in a tropical region. African Educational services. Owerri, Nigeria. 1999.

11. AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 14th ed. Washington, DC.; 1984.

12. AOAC. Official methods of analysis of the Association of Official Analytical Chemists. 2 vols. 15th ed. Washington, DC.; 1990.

13. Nwaoguikpe RN, Uwakwe AA. The antisickling effects of dried fish (tilapia) and dried prawn (Astacus red). 2005.

14. Plummer DT. An introduction to practical biochemistry. McGraw-Hill London. 1971.

15. Chao-Ming L, Ning-Hua T, Hui-Lan Z, Qing M, Xiao-Jiang H, Yi-Neng H, Jun Z. Cyclopeptide from the seeds of Annona muricata. Phytochemistry. 1998;48(3):555-6.

16. Agu KC, Okolie PN. Proximate composition, phytochemical analysis, and in vitro antioxidant potentials of extracts of Annona muricata (Soursop). Food science & nutrition. 2017;5(5):1029-36.

17. Egunyomi A, Moody JO, Eletu OM. Antisickling activities of two ethnomedicinal plant recipes used for the management of sickle cell anaemia in Ibadan, Nigeria. African Journal of Biotechnology. 2009;8(1).

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