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Original Research Article

Phytochemical and Nutritional Composition of Crude Powder and Ethanol Extract of *Annona muricata* Leaves

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ABSTRACT

Annona muricata (Soursop) is a tropical plant used in folk medicine in the treatment of different diseases and infections. In the present study, we investigated the phytochemical constituents and nutritional composition of *Annona muricata* leaves. Phytochemical screening was performed on the ethanol leaf extract of *A. muricata* using standard procedures. Proximate and mineral content of the leaves were also analysed. The result of the phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenoids, carbohydrates, proteins, phytosterols, and saponins while anthraquinones and reducing sugars were absent. The quantitative evaluation showed that the ethanol leaf extract contains a high amount of phenolic compounds such as proanthocyanidins, phenolics, and flavonoids. The leaves of *Annona muricata* were found to be rich in carbohydrate (27.81%), fat (21.19%), and moisture (21.15%). The total protein (12.69%) was considered to be of moderate value, while the ash (8.67%) and crude fibre (8.50%) were lesser. Minerals such as calcium (72.52 ppm), potassium (49.92 ppm), sodium (30.33 ppm), iron (25.8 ppm), magnesium (18.52 ppm), phosphorus (10.87 ppm) and zinc (2.63 ppm) were also detected. The result of this study shows that the leaves of *A. muricata* are rich sources of secondary active compounds of possible therapeutic value. It is also a good source of essential and non-essential nutrient required for metabolic processes in the body.

Keywords: *Annona muricata*, phytochemical screening, nutritional composition, drug discovery.

Introduction

For several decades, man has cultivated plants not only for food but also for medicinal purpose in the prevention and treatment of diseases.¹ In recent times, several parts of plants have been investigated for possible therapeutic property as well as potential sources of new drugs. Medicinal plants are of immense economic importance and are consumed around the world, particularly by the rural population of developing countries, including Nigeria.² These plants contain bioactive compounds and nutrients which are of health benefits to man. Phytochemicals are natural products of plants' secondary metabolism which accumulates in the roots, stems, leaves, flowers, fruits or seeds at varying degrees depending on the variety, growth and environmental conditions.³ Besides these bioactive secondary metabolites, medicinal plants possess nutrients consisting of various essential food classes. These proximates, vitamins, macro as well as trace element play vital roles in providing man with sufficient energy and other growth requirements.⁴

Annona muricata popularly called "sour-sop" is native to the Southern part of Nigeria.⁵ It belongs to the custard apple trees family, popularly known as "Annonaceae" with an oblong to oval-shaped leaves. The fruits are evergreen, firm and prickly on the side, and the spikes are much closer where the skin has not swollen.⁶ When ripe, the edible white fruit pulp has a slightly acidic flavour with a distinct aroma and is used in the preparation of beverages, candy, ice creams, shakes and syrups.^{2,6} All parts of the plant have found use as conventional therapy

for cancer, parasitic infections, and a range of diseases affecting man. In the humid regions of Africa such as Nigeria, the plant is used as an astringent, insecticide, pesticide, to suppress cough, relief pain and for the treatment of skin diseases.⁶ In addition, previous studies have reported that the leaves, barks and roots of *A. muricata* possess antioxidant, anti-inflammatory, anticancer, antidiabetic, hypolipidaemic, sedative, hypotensive and antispasmodic effects.^{1,6} These pharmacological properties are linked to its abundant phytochemical constituents which are acting individually, or in synergy to alleviate various health disorders.⁷

All *Annona species* including *A. muricata* have been shown to be a rich source of annonaceous acetogenin compounds (AGEs).⁸ These are a unique class of compounds derived from long chain (C-32/C-34) fatty acids in the polyketide pathway. Due to the special structures and extensive biological activities, AGEs have attracted significant scientific interest in recent years. However, the biological activities of AGEs are primarily characterized with toxicity against cancer cells via its inhibitory effects against the mitochondrial complex I (NADH: ubiquinone oxidoreductase) and cellular ATP production.⁹ Phytochemical investigations and biological studies on different parts of the *A. muricata* plant resulted in the identification of over 120 AGE compounds.^{8,9} The study attempt to exploit well-established scientific methods to identify the resident phytochemicals in the leaves of *A. muricata*. It also attempts to comparatively examine the different amounts of the phytochemicals in the crude and ethanol leaf extract, as well as its nutritional composition.

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Materials and Methods

Collection and preparation of plant materials

Fresh leaves of *Annona muricata* were purchased in July, 2020 from a local market in Benin City, Edo State, Nigeria, and identified by a Botanist in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City with the

voucher number UBH-A356. The leaves were washed and air-dried at room temperature for four weeks. The air-dried leaves were then pulverized, weighed and a small portion of the crude powdered leaves was used for proximate and mineral analyses. The ethanol extract was prepared by macerating 100 g of the pulverized plant sample in 1700 mL of absolute ethanol at room temperature for 72 hours. The extract was then filtered, the filtrate was concentrated using a rotary evaporator, and then freeze-dried. The dried residue (ethanol extract) was stored at 4°C. The extract was later reconstituted to obtain a stock solution (1 mg/mL) using distilled water as solvent, and used for phytochemical screening.

Qualitative phytochemical screening

Qualitative tests for alkaloids, flavonoids, tannins, saponins, anthraquinones, phenols, carbohydrates, reducing sugar, protein, phytosterol and terpenoids were performed according to the procedure described by Tiwari *et al.*¹⁰

Quantitative phytochemical analysis

Phytochemical screening was performed for the quantitative determination of the total phenolic, flavonoid and tannin content of the ethanol leaf extract of *A. muricata*. The method used was described by Kavitha and Indira.¹¹ The total proanthocyanidin content was determined using the procedure described by Sharma and his colleagues.⁷

Determination of proximate composition

The dry matter, moisture, ash, crude fat, crude protein, carbohydrate and crude fibre contents of the crude powdered leaves of *A. muricata* were determined using the standard methods of the Association of Official Analytical Chemists (AOAC).¹²

Determination of mineral content

The AOAC (2000) method was used to determine the mineral content. Sodium (Na) and potassium (K) levels of the crude powdered leaves of *A. muricata* were ascertained using a flame photometer. Other metals such as calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe) and Zinc (Zn) were determined using atomic absorption spectrometric method.¹³

Statistical analysis

Data were expressed as mean \pm S.E.M. Data were subjected to one-way analysis of variance (ANOVA) using windows Statistical Package for Social Science (SPSS) package. Post hoc testing was performed, and P-values < 0.05 was considered statistically significant.

Results and Discussion

Qualitative phytochemical screening

The result of the qualitative phytochemical screening is presented in Table 1. The phytochemical screening revealed the presence of alkaloids, flavonoids, tannins, terpenoids, carbohydrates and proteins. Other plant metabolites such as phytosterols, saponins, and phenols were also present, while anthraquinones and reducing sugars were absent. Our findings are similar to that obtained by previous investigators except for anthraquinones,¹⁴ saponins,² and flavonoids.¹⁵ Flavonoids and phenols possess antioxidant and antitumor properties by preventing free-radicals mediated oxidative damage to cells as well as inhibit the processes underlying tumour formation.⁵ The analgesic and stimulatory potency of alkaloids have been long established, and they are also known for their ability to repel insect. Steroids are known for their anti-inflammatory property, saponins for cholesterol-lowering and maintenance of membrane integrity, and finally tannins for its astringency.¹⁴

Quantitative phytochemical analysis

The result of the quantitative phytochemical screening is presented in Figure 1. The quantitative evaluation showed that the ethanol extract of *Annona muricata* leaves are rich in proanthocyanidins, followed by flavonoids and phenolics. In contrast, the total tannin content was

found to be quite low. This result implies that ethanol can serve as a suitable solvent for the extraction of phenolic compounds such as flavonoids, proanthocyanidins, etc due to its amphipathic nature. Besides, ethanol has been found to absorb intracellular ingredients easily across the membrane of plant materials.¹⁰ This have also been ascribed to the inhibition of the activity of the enzyme polyphenol oxidase by ethanol, which degrades polyphenols in the presence of water. Hence making ethanol a more suitable solvent.¹⁰

Proximate composition of *A. muricata* leaves

The result of the proximate analysis of the leaves of *Annona muricata* is presented in Figure 2. Proximate analysis of the leaves of *Annona muricata* was aimed at establishing the role of its dietary constituents in its therapeutic uses. The result obtained showed that *Annona muricata* leaves contain fibre (8.50%), ash (8.67%) and protein (12.69%). However, it is notably rich in carbohydrate (27.81%), fat (21.19%) and moisture (21.15%). Carbohydrate metabolism ensures a steady supply of energy to living cells as well as provide intermediates that fuels other biochemical processes such as lipogenesis, amino acid synthesis as well as nucleotide biosynthesis.¹⁶ Dietary fats are not just a source of energy; they also function as insulators, aid absorption of fat-soluble vitamins, and are required for growth, immune function and reproduction. However, the intake of high-fat diet has been positively correlated with obesity and the risk of development of cardiovascular disorders.¹ Proteins in diet serve as an alternative source of energy; they are also required for building and repair of body tissues, synthesis of enzymes, hormones, antibodies and other materials needed for healthy functioning.⁵ The high moisture content shown in this study suggest that the plant leaf may be prone to microbial attack during storage resulting in decreased shelf life.¹⁶ The ash content is a reflection of the mineral contents preserved in the plant leaf. Hence, the result indicates that the leaves of *A. muricata* may contain more of the organic components as seen in its low ash value. Dietary fibre improves gut motility and plays a role in the prevention of coronary heart disease, diabetes and cancer.¹³

Mineral content of *A. muricata* leaves

The result of the mineral content of *A. muricata* leaves is summarised in Figure 3. The result of the mineral analysis showed that the leaves of *Annona muricata* are rich in essential and trace elements, with calcium, potassium, sodium, iron and magnesium being the most abundant. This result was found to be in consonance with that obtained by previous investigators.^{13,17} Calcium is central to blood clotting mechanism, muscle contraction, hormone regulation and together with phosphorus, it aids the formation of bones and teeth. In addition, phosphorus is a vital component of the phosphate backbone of nucleic acids and the polar head of membrane phospholipids.¹³

Table 1: Phytochemical constituents of *A. muricata* leaves

Phytochemicals	Ethanol extract
Phytosterol	+
Saponins	+
Tannins	+
Phenol	+
Flavonoids	+
Anthraquinones	-
Terpenoids	+
Carbohydrate	+
Reducing sugar	-
Protein	+
Alkaloids	+

Key: + = present; - = absent

Potassium and sodium are essential electrolytes found in biological fluids and are central to the maintenance of cellular membrane potential, osmoregulation as well as the electrical excitation of nerve and muscle cells. Their deficiency has been associated with impaired renal function, alterations of gastric secretions and intestinal motility.¹⁶ Magnesium, iron and zinc are cofactors required for enzyme catalysis in various metabolic processes such as glycolysis, oxidative phosphorylation and nucleic acid synthesis. Also, iron is a crucial component of the oxygen-transporting protein “haemoglobin” whose deficiency leads to anaemia.^{17,18}

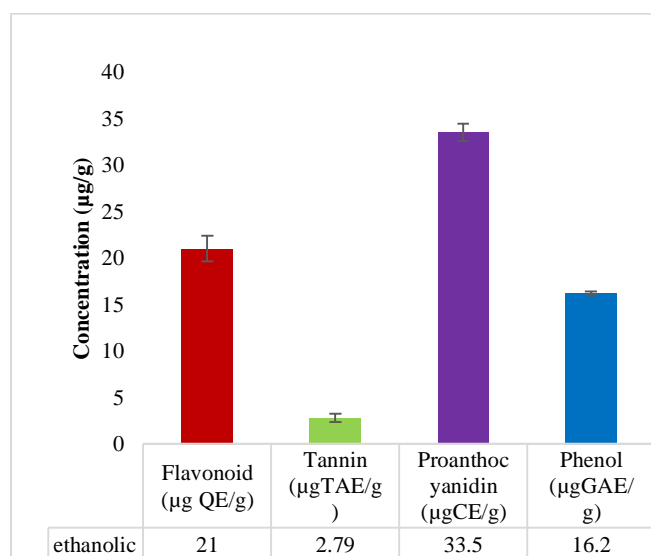


Figure 1: Quantitative phytochemical constituents of ethanol extract of *A. muricata* leaves.

Values are expressed as Mean \pm S.E.M.; n = 2. Bars with different letters are significantly different. Key: QE = Quercetin equivalent, TAE = Tannic acid equivalent, CE = Catechin equivalent, GAE = Gallic acid equivalent.

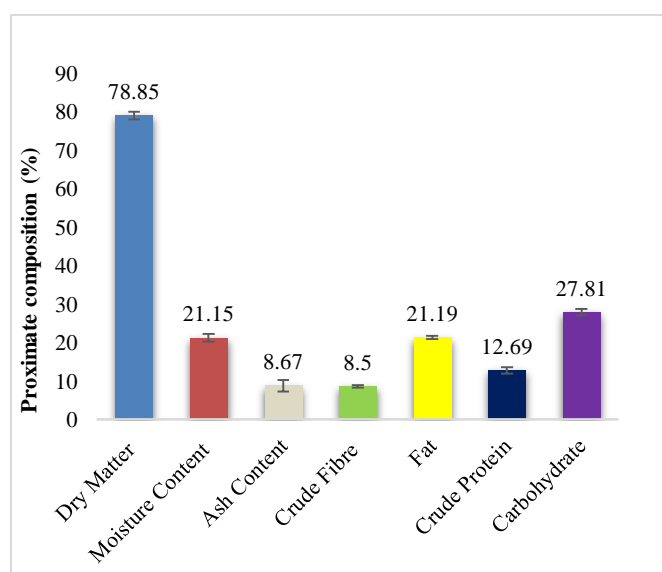


Figure 2: Proximate composition of *A. muricata* leaves.

Values are expressed as Mean \pm S.E.M.; n = 3.

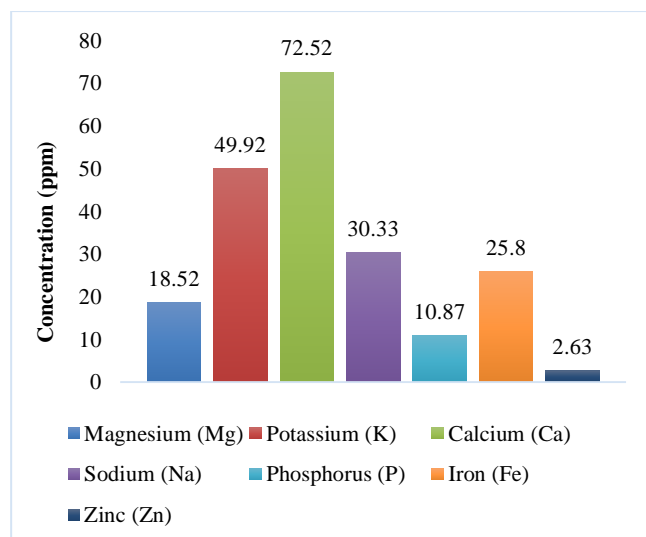


Figure 3: Mineral Content of *A. muricata* leaves

Conclusion

A. muricata leaves contain bioactive compounds like alkaloids, flavonoids, tannins, terpenoids, phytosterols, saponins, and phenols in both the crude and ethanol extract. Also, preparation of the plant extract with the use of ethanol should be encouraged. Proximates and mineral present in the leaves of *A. muricata* were found to be nutritionally adequate. Hence, this study implies that *A. muricata* is one of the richest store of novel therapeutic agent and biopharmaceuticals, as well as other nutritional and food supplement.

Conflict of interest

The authors declare no conflicting interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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