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PHYTOCHEMICAL SCREENING AND PROXIMATE COMPOSITION OF *ANNONA MURICATA* LEAVES

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ABSTRACT: *Proximate composition and phytochemical analyses were carried out on the leaves of Annona muricata using standard methods. The result of the proximate composition showed that the leaves contained 88.99% dry matter, 11.01% moisture, 25% crude protein, 14.96% ash, 22.20% crude fiber, 21.22 % fat and 16.62% carbohydrate contents. The phytochemicals detected in the ethanolic leaf extracts were flavonoids, alkaloids, cardiac glycoside, tannins, triterpenoid, saponin and reducing sugar. The findings indicate that Annona muricata leaves is a potential source of highly nutritious feed stuff and phytomedicine. They are of nutritional, clinical and veterinary relevance considering the diverse ethnopharmacological uses of the plant in different parts of the world.*

KEYWORDS: *Annona muricata*, phytochemicals, proximate, leaves, ethanol

INTRODUCTION

In recent years, there has been a gradual revival of interest in the use of medicinal plants in developing countries because herbal medicines have been reported safe and without any adverse side effect especially when compared with synthetic drugs. Thus, the search for new drugs with better and cheaper substitutes from plant origin is a natural choice. The medicinal value of these plants lies in some chemical substances that produce a definite physiological action on the human body (Edeoga *et al*., 2005). *Annona muricata* commonly called Soursop is commonly found in southern part of Nigeria. It is mostly eaten as fresh fruits. It is cultivated mainly in home gardens. The tree yields up to 10 tons per *ha* and each fruit weighs 0.5 to 2 kg (Oyenuga, 1978).

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Soursop has found its uses in many areas. It is consumed as a desert fruit. It is made into a fruit jelly with the addition of some gelatin or used in the preparation of beverages, ice creams and syrups. Its white edible pulp contains 80% water, 1% protein, 18% carbohydrate and fair amounts of vitamin B_1 and B_2. The seeds are flat, hard and contain all that can be used for paint or insecticide (Rice *et al.*, 1991). A number of medicinal properties are attributed to the leaves and juice of the soursop. The seed is about 4% of the whole fruit and much has not been reported about the chemical and nutritional contents of the leaves in recent times. This study is therefore conducted to determine selected nutritional and phytochemical properties of the leaves of *Annona muricata*.

MATERIALS AND METHODS

Collection, Identification and Preparation of *Annona Muricata* Leaves

Fresh leaves of *Annona muricata* were purchased from a local market in Benin City, Edo state, Nigeria and identified by Dr. Chris Akoma, a Botanist in the Department of Basic Sciences, Faculty of Basic and Applied Sciences, Benson Idahosa University, Benin city, Edo State. The *Annona muricata* leaves were separated from the stalk, washed and air-dried at room temperature (24^{o}C) and then pulverized, crushed into fine powder and weighed. Aliquot portions of the powdered leaves were weighed and used for proximate analysis.

Extraction of the Plant Leaves

Ethanolic extract of the plant leaves was prepared by soaking 100g of the dry powdered plant leaves in 1000ml of absolute ethanol at room temperature for 48hrs (for thorough extraction). The extract was then filtered first through a Whatmann filter paper No. 42 (125mm) and then through cotton wool. The extract was thereafter concentrated using a rotary evaporator with the water bath set at 60◦C to one-tenth its original volume and then finally freeze dried. The dried residue (crude extract) was then stored at 4ºC. Aliquot portions of the crude plant extract residue were weighed and used for phytochemical screening

Methods for Phytochemical Screening

Phytochemical screening was performed using standard procedures (Sofowora, 1993, Trease and Evans, 1989, Ayoola *et al.*, 2008).

Test for Saponins

0.5g of extract was added to 5ml of distilled water in a test tube and the solution was shaken vigorously and observed for a stable persistent froth. The frothing was mixed with 3 drops of olive oil and shaken vigorously after which it was observed for the formation of an emulsion.

Test for Triterpenoids

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0.5g of the extract was dissolved in 1ml of chloroform. 1ml of acetic anhydride was added, followed by the addition of 2ml of concentrated H_2SO_4. Formation of reddish violet colour indicates the presence of triterpenoids.

Test for Tannins
Two methods were used to test for tannins:
(a) To 10ml of freshly prepared 10% KOH in a beaker, 0.5g of extract was added and shaken to dissolve. A dirty precipitate observed indicated the presence of tannin.

(b) About 0.5g of the extract was boiled in 10ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and the solution observed for brownish green or a blue-black colouration

Test for Reducing Sugar (Fehling's Test)
0.5g of the extract was dissolved in 5ml distilled water and filtered. The filtrate was hydrolysed with dilute HCl, neutralized with alkali (NaOH) and heated with Ferling`s A and B solutions. Formation of red precipitate indicated the presence of reducing sugars.

Test for Anthraquinones
0.5g of the extract was boiled with 10ml of H_2SO_4 and filtered while hot. The filtrate was shaken with 5ml of chloroform, the chloroform layer was pipette into another test tube and 1ml of dilute ammonia was added. The resulting solution was observed for colour changes.

Test for Steroids
0.5g of the extract was dissolved in 10ml of chloroform and equal volume of concentrated H_2SO_4 was added by the sides of the test tubes. Reddish upper layer and yellowish sulphuric acid layer with green fluorescence indicate the presence of steroids.

Test for Cardiac Glycosides (Keller-Killiani Test)
To 0.5g of extract dissolved in 5ml water was added 2ml of glacial acetic acid solution containing one drop of ferric chloride solution. This was underlayed with 1ml of concentrated H_2SO_4. A brown ring at the interface indicated the presence of a deoxysugar characteristics of cardenolides. A violet ring may appear below the brown ring while in the acetic acid layer a greenish ring may form just above the brown ring and gradually spread throughout this layer.

Test for Flavonoids
Two methods were used to test for flavonoids:
(a) A portion of the extract was heated with 10ml of ethyl acetate over a steam bath for 3 minutes, the mixture was filtered and 4ml of the filtrate was shaken with 1ml of dilute ammonia solution. A yellow colouration indicated the presence of flavonoids.

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(b) Dilute ammonia (5ml) was added to a portion of an acqueous filtrate of the extract. Then, concentrated sulphuric acid (1ml) was added. A yellow colouration indicated the presence of flavonoids.

Test for Alkaloids

Extracts were dissolved individually in dilute HCl and filtered.

(a) filtrates were treated with Mayer`s reagent (potassium mercuric iodide). Formation of a yellow coloured precipitate indicates the presence of alkaloids.

(b) Filtrate was treated with Dragendroff`s reagent (solution of potassium bismuth iodide). Formation of red precipitate indicates the presence of alkaloid.

Filtrate was treated with Hager`s reagent (saturated picric acid solution). Presence of alkaloid is confirmed by the formation of yellow coloured precipitate.

METHODS FOR PROXIMATE ANALYSIS

The powdered leaves were taken for proximate analysis. The dry matter, moisture, ash, crude fat, crude protein (nitrogen x 6.25) and crude fibre contents were determined using the standard methods of the Association of Official Analytical Chemists (AOAC, 2000). Carbohydrate content was estimated based on the net difference between the other nutrients and the total percentage composition.

RESULTS

The results of the phytochemical screening of the ethanolic leaf extract of *Annona muricata* showed the presence of various secondary metabolites such as flavonoids, alkaloids, saponins, tannins, triterpenoids, reducing sugars and cardiac glycosides (Table 1). These finding suggest that this plant is a potential source of natural antioxidants that could serve great importance as therapeutic, anti-inflammatory, anti-analgesic and anti-hyperlipidemic agent.

Table 1: Phytochemical screening of *Annona muricata* ethanolic leaf extract

Pytochemicals	***Annona muricata***
Flavonoids	+ve
Saponins	+ve
Tannins	+ve
Steroids	-ve
Alkaloids	+ve
Triterpenoids	+ve
Anthraquinone	-ve
Reducing sugar	+ve

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Cardiac glycosides	+ve

Where; +ve = present, -ve =absent

The result in Table 2 indicate that the leaves of *Annona muricata* in percentage (%) are rich in carbohydrates (16.62 ± 0.09), proteins (25±0.06), fibers (22.20 ± 0.05), ash (14.96 ± 0.05), fats/oil (21.22 ± 1.01), dry matter (88.99±0.74) and moisture content (11.01 ± 0.82).

Table 2: Proximate analysis of *Annona muricata* powdered leaves

Proximate composition	***Annona muricata* (%)**
Dry matter	88.99 ± 0.74
Moisture Content	11.01 ± 0.82
Crude protein	25.00 ± 0.06
Crude fibre	22.20 ± 0.05
Ash content	14.96 ± 0.05
Crude fat/oil	21.22 ± 1.01
Carbohydrate	16.62 ± 0.09

Values are means ± SD for 3 determinations.

DISCUSSION

Many common plants based foods and herbs contain powerful phytochemical substances that can improve the quality of our health. Phytochemicals protect us against many diet related diseases. Results of the phytochemical screening of *Annona muricata* ethanolic leaf extract showed the absence of steroid and anthraquinone while flavonoids, saponins, tannins, alkaloids, triterpenoids, reducing sugar and cardiac glycosides were present. These phytochemicals exhibit various pharmacological and biochemical actions when ingested by animals. Plants used in the treatment of diseases are said to contain bioactive principles with biological activity some of which are responsible for the characteristic odor, pungencies and color of plant, while others give the particular plant its culinary, medicinal or poisonous virtue (Evans, 2002). The qualitative phytochemical screening of *Annona muricata* was in agreement with the works of Foong and Hamid, (2012), Falodun *et al*, (2011), and Vijayameena *et al* (2013).

It has been reported that flavonoids and phenolics are free radical scavengers that prevent oxidative cell damage, and have strong anticancer activities (Pourmorad *et al*., 2006; Ugwu *et al*., 2013) and they might induce mechanism that affect cancer cells and inhibit tumor invasion (Rafat *et al.*, 2008). These activities could be attributed to their ability to neutralize and quench free radicals (Ugwu *et al*., 2013; Pourmorad *et al*., 2006; Omale and Okafor, 2008). It can also

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be due to their redox properties, presence of conjugated ring structures and carboxylic group which have been reported to inhibit lipid peroxidation (Rice-Evans *et al*., 1995).

Herbs that have tannins as their component are astringent in nature and are used for the treatment of intestinal disorders such as diarrhoea and dysentery (Bajai, 2001), thus supporting the reasons why *Annona muricata* has position among medicinal plants used for the treatment of microbial infection. Tannins are known to be useful for the prevention of cancer as well as treatment of inflamed or ulcerated tissues. (Okwu and Emineke, 2006; Li *et al*., 2003; Adegboye *et al.*, 2008). Alkaloids are beneficial chemicals to plants serving as repellant to predators and parasites. This probably endows these group of agents its antimicrobial activity. Several alkaloid containing medicinal plants are reported to have been used by the early man as pain relievers, as recreational stimulants or in religious ceremonies to enter a psychological state to achieve communication with ancestors or God (Heinrich *et al*., 2005; Gurib-Fakin, 2005).Saponins are believed to react with the cholesterol rich membranes of cancer cells, thereby limiting their growth and viability (Roa *et al.*, 1995). Saponins have the property of precipitating and coagulating red blood cells (Yadav and Agarwala, 2011). Some of the characteristics of saponins include formation of foams in aqueous solutions, hemolytic activity, cholesterol binding properties and bitterness (Sodipo *et al.*, 2000; Okwu, 2004). Saponins in medicinal plants are responsible for most biological effects related to cell growth and division in humans and have inhibitory effect on inflammation (Just *et al.*, 1998; Okwu and Emineke, 2006, Liu and Henkel, 2002).

Cardiac glycosides are important class of naturally occurring drugs whose actions helps in the treatment of congestive heart failure (Yukari *et al*., 1995). *Annona muricata* is used for the treatment of cardiac infections along with other ailments such as cough, and chest pain in Jamaica, Haiti, and the West Indies (Technical Data Report for Graviola, 2005; Taylor, 2002)
Though several works reporting compositional evaluation and functional properties of various types of edible wild plants in use in the developing countries abound in literature, much still need to be done. Dietary fiber, polyunsaturated fatty acids (PUFA), proteins, amino acids, minerals, vitamins and other bioactive compounds are considered as beneficial nutrient components (Andlauer and Fürst, 2002). The nutrient composition in this study revealed that *Annona muricata* leaves contained protein, fiber, ash, fats/oil as well as carbohydrate as shown in Table 2. Our result suggests that *Annona muricata* leaves could serve as better sources of dietary carbohydrate, protein and lipids. Hence *Annona muricata* leaves add to the calorific value of food and possess odour and flavor carrying ability thereby enhancing the palatability of food. There is scarce data on proximate analysis in *Annona muricata* leaves.

The leaves of *Annona muricata* contained crude protein value of 25% which is higher than protein content of *Momordica foecide* (4.6%) leaves consumed in Nigeria and Swaziland, *Lesianthera africanas* (13.1%) (Hassan and Umar, 2006; Ogle and Grivetti, 1985; Isong and Idiong, 1997), *Amaranthus candatus* (20.5% DW), but lower than *Piper guineeses* (29.78% DW)

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and *T. triangulare* (31.00% DW) (Akindahunsi and Salawu, 2005; Antia *et al*., 2006; Etuk *et al*., 1998). High amount of protein is essential for animal growth and increased milk production (Tangka, 2003). Plant proteins are a source of food nutrient especially for the less privileged population in developing countries including Nigeria. Proteins are one of the macromolecule and it is an alternate energy source when other energy sources are in short supply. They are building block units and food protein is needed to make vital hormones, important brain chemicals, antibodies, digestive enzymes, and necessary elements for the manufacture of DNA. Some proteins are involved in structural support, while others are involved in bodily movement, or in defense against germs (Bailey, 2008). *Annona muricata* leaves can thus be considered a good source of protein because they provide more than 12% of caloric value from protein. Therefore, the protein content of the *Annona muricata* leaves will go a long way in meeting the protein requirement of the local people.

The leaves of *Annona muricata* contained crude fibre value of 22.20% which is high when compared to *Ipomea batatas* (7.20%), *T. triangulare* (6.20%) *P. guineensis* (6.40%), and *Corchorus olitorius* (7.0%), (Akindahunsi and Salawu, 2005; Antia *et al.*, 2006). Fibre cleanses the digestive tract by removing potential carcinogens from the body and prevents the absorption of excess cholesterol. Fibre also adds bulk to the diet and prevents the intake of excess starchy food (Mensah *et al.*, 2008) and may therefore guard against metabolic conditions such as hypercholesterolemia and diabetes mellitus (Henry, 2004). Dietary fiber has a positive effect in the management of diabetes by controlling post-prandial hyperglycemia. It delays gastric emptying or increase the viscosity of gastro-intestinal tract content thereby suppressing digestion of carbohydrate and delays its absorption. The substantial amount of fibre in *Annona muricata* leaves shows that they can help in keeping the digestive system healthy and functioning properly. Fibre aids and speeds up the excretion of waste and toxins from the body, preventing them from sitting in the intestine or bowel for too long, which could cause a build-up and lead to several diseases (Hunt *et al*., 1980). Adequate intake of dietary fibre can lower the serum cholesterol level, risk of coronary heart disease, hypertension, constipation, diabetes, colon and breast cancer (Rao and Newmark, 1998; Ishida *et al.*, 2000). The RDA of fibre for children, adults, pregnant and lactating mothers are 19 – 25, 21-38, 28 and 29 g, respectively (Jimoh *et al*., 2011).

Annona muricata had ash value of 14.96% and the ash content is a reflection of the mineral contents preserved in the plants leaf. The ash content of *Annona muricata* leaves compare favorably with the values reported for *Vernonia colorate* (15.86%) and *Moringa oleifera* (15.09%) (Lockeett *et al*., 2000; Antia *et al*., 2006) and lower than that of some leafy vegetables commonly consumed in Nigeria such as *Talinum triangulare* (20.05%) but higher than some other vegetables such as *Occimum graticimum* (8.00%) and *Hibiscus esculentus* (8.00%) (Akindahunsi and Salawu, 2005). The result therefore suggests a high deposit of mineral elements in *Annona muricata* leaves.

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Annona muricata had carbohydrate value of 16.62%, which is lower than reported values for *Corchorus tridens* (75.0% DW) and sweet potatoes leaves (82.8%) (Asibey-Berko and Taiye, 1999). Thus the carbohydrate content contributes to the energy value in *Annona muricata*. Carbohydrates are essential for the maintenance of life in both plants and animals and also provide raw materials for many industries (Ebun-Oluwa and Alade, 2007). Carbohydrates produced by plants are one of the three main energy sources in food, along with protein and fat. When animals eat plants, energy stored as carbohydrates is released by the process of respiration, a chemical reaction between glucose and oxygen to produce energy, carbon dioxide, and water. Glucose is also used by animal cells in the production of other substances needed for growth (Westman, 2002).

Annona muricata had a crude fat value of 21.22%, thus contributes to the energy value of *Annona muricata*. Dietary fat increases the palatability of food by absorbing and retaining flavours (Antia *et al*., 2006).
The moisture content value of *Annona muricata* leaves was relatively low. The low moisture content would therefore hinder the growth of spoilage microorganisms and enhance shelf life (Ruberto and Baratta, 2000).

This study shows that *Annona muricata* leaves are rich in phytochemicals and that their utilization should be strongly recommended for good health. *Annona muricata* leaves are reservoirs for free radical scavenging molecules such as vitamins, alkaloids, tannins, terpenoids, phenolic acids, flavonoids and other metabolites, which are basically rich in antioxidant activities. It is quite interesting to inform that these plant metabolites can be genetically manipulated to increase their yield. Additionally, the DNA copy or gene(s) responsible for the expression of these metabolites could be cloned and inserted into other edible crops for ease of consumption by end users. This implies that it might be unnecessary to go over the counter medicine stores to buy synthetic drugs to this respect.

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