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PHYTOCHEMICAL ANALYSIS AND MINERAL COMPOSITION OF ANNONA MURICATA LEAVES

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ABSTRACT

Many local vegetable materials are under-exploited because of inadequate scientific knowledge of their nutritional potentials. For this reason, the phytochemicals and mineral composition of the leaves of *Annona muricata* obtained from Benin City, Edo state, Nigeria were investigated. The mineral analysis was done using Atomic Absorption Spectrophotometric analysis method while phytochemical screening was determined using standard methods. The result of the phytochemical analysis shows the following: saponins (3.50%), alkaloids (1.20%), flavonoids (9.67%), tannins (0.18%), beta-carotene (6.60mg/100g), ascorbic acid (38.16mg/100g) and reducing sugars (48.33%) The result also shows that the mineral concentrations are as follow: potassium (363.05mg/kg), calcium (11183.50mg/kg), sodium (694.86mg/kg), magnesium (9619mg/kg), iron (139.50mg/kg), zinc (8.34mg/kg), manganese (8.25mg/kg), chromium, (3.75mg/kg), copper, (14.25mg/kg), cadmium (5.49mg/kg). The results obtained from the analysis shows that the leaves of *Annona muricata* are medicinal and could be good source of drugs for anemia because of the high contents of iron and calcium.

INTRODUCTION

Annona muricata, commonly known in English speaking countries as 'soursop' and '*ebo*' in Yoruba, is an upright, low-branching tree reaching 8 to 10 meters (popenoe, 1920; Mowry *et al*., 1953; Morton, 1987). The tree has green, glossy evergreen leaves, and the flowers appear anywhere on the trunk or any branch (Morton, 1966). Traditionally, the leaves are used for headaches, insomnia, cystitis, liver problems, diabetes, hypertension and as an anti-inflammatory, anti-spasmodic and anti-dysenteric (Di Stasi and Hiruma-Lima, 2002; Sousa *et al.*, 2004). In the West Indies, various parts of the plant, including the leaves, bark and roots have been used to treat disease conditions such as diabetes (Adeyemi *et al.*, 2008, 2010) and arthritis. Other reported medicinal uses of *Annona muricata* include its anticancer (Oberlies *et al.*, 1997; Liaw *et al.*, 2002), antibacterial and antifungal actions, as well as, its antinociceptive and anti-inflammatory effects (de Sousa *et al.*, 2010). The plant has been reported to possess acetogenins as major phytoconstitutents (Padma *et al.*, 1996) which are responsible for a number of activities such as antitumor, immunomodulator, anti-spasmodic, anti-malarial, pesticidal, anti-parasitic, anti-bacterial, anti-fungal and anti-helmintic activity (Padma, 1997). This study is aimed at determining the quantitative phytochemicals and mineral composition of *Annona muricata* leaves.

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MATERIALS AND METHODS

Collection and preparation of plant leaves

Fresh leaves of *Annona muricata* were collected from the tree in Ugbowo 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°C) and then pulverized, crushed into fine powder and weighed. Aliquot portion of the powdered leaves were weighed and used for Mineral and quantitative phytochemical analysis.

Methods for quantitative phytochemical analysis

Alkaloid was determined using the method of Harborne (1973), tannin using the Van–burden and Robinson (1981) method, saponin using the method of Obadoni and Ochuko (2001), flavonoid using Boham and Kocipai-abyazan (1994) method, beta-carotene according to Alexander and Griffiths (1993) method, vitamin-C content according to the method of Adebayo (2010) while reducing sugar was determined according to the method of Bertrand *et al* (2003)

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Mineral analysis using Atomic absorption spectrophotometer (AAS)

An acid digest of the powdered leaf was prepared by oxidizing 0.2g of the sample with conc. HCl/nitric acid followed by kjeldahl heating at 70°C until brown fumes disappeared. The digest was diluted with distilled water and heated again (until colourless solution is obtained) to a certain volume and thereafter filtered with Whatmann filter paper No. 1 (110mm). The filtrate was then made up to 100ml with distilled water. Aliquots were thereafter used for mineral analysis using the Atomic Absorption Spectrophotometer (AAS). The blank and working standards were first run followed by the samples. Each sample was analysed twice, and the data reported as a mean of the analysed samples in mg/kg.

Statistical Analysis

Results obtained were expressed as mean ± standard deviation.

RESULT

The quantitative result of the phytochemicals shown in Table 1 revealed that the leaves of *Annona muricata* is rich in saponins (3.50%), alkaloids (1.20%), flavonoids (9.67%), tannins (0.18%), beta-carotene (6.60mg/100g), ascorbic acid (38.16mg/100g) and reducing sugars (48.33%).

The result of mineral elements found in the leaf of *annona muricata* shown in Table 2 are as follows: potassium (363.05mg/kg), calcium (11,183.50mg/kg), sodium (694.86mg/kg), magnesium (9,619mg/kg), iron (139.50mg/kg), zinc (8.34mg/kg), manganese (8.25.00mg/kg), chromium (3.75mg/kg), copper (14.25mg/kg) and cadmium (5.49mg/kg). Calcium was found to have the highest concentration and chromium the least in concentration.

Table 1 Phytochemical analysis of *Annona muricata* powdered leaves

Phytochemicals	*Annona muricata*
Flavonoids	9.67 ± 0.07 %
Saponins	3.50 ± 0.40 %
Alkaloids	1.20 ± 0.05 %
Beta-carotene	6.60 ± 0.80mg/100g
Ascorbic acid	38.16 ± 1.94mg/100g
Reducing sugar	48.33 ±1.20%
Tannins	0.18 ± 0.07%

Values are means ± SD for 3 determinations.

Table 2 Mineral analysis on *Annona muricata* powdered leaves (mg/kg)

Metals	*Annona muricata* (mg/kg)
Sodium	694.86±10.65
Potassium	363.05±3.46
Magnesium	9619±801
Calcium	11183±10
Zinc	8.34±0.56
Manganese	8.25±1.25
Iron	139.50±32
Copper	14.25±0.75
Chromium	3.75±0.20
Cadmium	5.49±0.07

Values are means ± SD for 2 determinations.

DISCUSSION

The phytochemical analysis conducted on *Annona muricata* dried and powdered leaves (Table 1) revealed the quantitative percentage of ascorbic acid, beta-carotene, flavonoids, alkaloids, tannins, saponins and reducing sugars. These phytochemicals are known to support bioactive activities in medicinal plants and are thus could be responsible for antioxidant activities of this plant. Tannins are known to be useful in the treatment of inflamed or ulcerated tissues and have remarkable activity in cancer prevention (Ruch *et al.*, 1989, Motar *et al.*, 1985). Thus *Annona muricata* containing tannins may serve as a potential source of bioactive compound in cancer prevention and treatment. Flavonoids are potent water soluble antioxidants and free radical scavengers, which prevent oxidative cell damage and have strong anticancer activity (Salah *et al*, 1995, Del-Rio *et al*, 1997, Okwu, 2004). It has been recognized that flavonoids, which contain hydroxyl groups are responsible for the radical scavenging effects of most plants. Alkaloids are beneficial chemicals to plants, serving as repellant to predators and parasites. Alkaloids have been found to have microbiocidal effect and the major anti-diarrheal effect is probably due to their effects on small intestine and anti-hypertensive effect (Trease and Evans, 1985). Some alkaloids are useful against HIV infection as well as intestinal infection associated with AIDS (McDevitt *et al*., 1998). Also, *Annona muricata* leaves was revealed to contain saponins, known to produce inhibitory effect on inflammation (Just *et a.l.*, 1998). Saponins as a class of natural products are involved in complexation with cholesterol to form pores in cell membrane bilayers (Francis *et al*., 2002), and as such may be used as anti-cholesterol agents or cholesterol lowering agent. The presence of these phenolic compounds in *Annona muricata* leaves contributes to their antioxidative properties and thus the usefulness of these plants in herbal medicament.

Ascorbic acid also known as vitamin C is a well-known antioxidant that helps to strengthen immune system by reducing the amount of free radicals in the body (Iyawe and Onigbinde, 2009) and suppression of peroxidation in both aqueous and lipid region of cells (Gora *et al*., 2006). Lack of ascorbic acid impairs the normal formation of intercellular substances throughout the body, including collagen, bone matrix and tooth dentine. A striking pathology resulting from this defect is the weakening of the endothelial wall of the capillaries due to a reduction in the amount of intercellular substances (Hunt *et al*., 1980). Therefore, the clinical manifestations of scurvy hemorrhage from mucous membrane of the mouth and gastrointestinal tract, anemia, pains in the joints can be related to the association of ascorbic acid and normal connective tissue metabolism (Okwu, 2004). Ascorbic acid boosts immunity, fights against infection and improves iron absorption from food. It traps peroxyl radicals before they can initiate lipid peroxidation and helps in the regeneration of Vitamin E (Chatterjee and Nandhi, 1991). Vitamin C acts as a strong antioxidant in the plasma, and may present a synergistic effect with other antioxidants or coantioxidants.

Mineral elements serve as structural components of tissues and as constituents of the body fluid and vital enzymes in major

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metabolic pathways and are essential for the function of all cells (Lozak and Ostapezuk, 2002). The mineral composition of *Annona muricata* powdered leaves in mg/kg is as shown in Table 2 with calcium having the highest concentration and chromium the least. Other mineral revealed includes potassium, sodium, iron, magnesium, zinc, copper, cadmium and manganese. Sodium is involved in the regulation of plasma volume, acid-base balance, nerve and muscle contraction (Akpanyung, 2005). Sodium remains one of the major electrolytes in the blood. Without sodium the body cannot be hydrated, as it would dry up. Calcium is necessary for the coagulation of blood, the proper functioning of the heart and nervous system and the normal contraction of muscles. Its most important function is to aid in the formation of bones and teeth. Magnesium is a component of chlorophyll and it is an important mineral element in connection with ischemic heart disease and calcium metabolism in bones (Ishida *et al*., 2000). Iron is an essential element for hemoglobin formation, normal functioning of the central nervous system and oxidation of carbohydrate, protein and fats (Adeyeye and Otokili, 1999). Iron plays crucial roles in haemopoiesic, control of infection and cell mediated immunity (Bhaskaran, 2001). The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide (Trowbridge and Martorell, 2002). The consequences of iron deficiency include reduced work capacity, impairments in behaviour and intellectual performance and decrease resistance to infection (Dioxin and Harris, 2004). Zinc is involved in normal function of immune system and is a component of over 50 enzymes in the body (Okaka *et al*., 2006). An estimated 20% of the world population is reported to be at risk of inadequate zinc intake (Hotz and Brown, 2004). Studies on Nigerian shows that zinc deficiency affects 20% of children less than five years, 28.1% of mothers and 43.9% of pregnant women (Dioxin and Harris, 2004). Potassium is the principal cation in intracellular fluid and functions in acid-base balance, regulation of osmotic pressure, conduction of nerve impulse, muscle contraction particularly the cardiac muscle, cell membrane function and Na^+/K^+-ATPase. Deficiency disease or symptoms occurs secondary to illness, functional and structural abnormalities including impaired neuromuscular functions of skeletal, smooth, and cardiac muscle, muscular weakness, paralysis, mental confusion (Hays and Swenson, 1985; Murray *et al*., 2000). Potassium deficiency affects the collecting tubules of the kidney, resulting in the inability to concentrate urine, and also causes alterations of gastric secretions and intestinal motility (Streeten and Williams, 1952).

Immune cells, like all other types of cells, require an adequate supply of trace elements (Fe, Cu and Zn) to express and preserve the structure and function of key metalloproteins that participate in housekeeping processes such as energy production and to protect the cell against highly toxic ROS. Also adequate level of Fe and Zn is required for continous generation of immune cells in bone marrow and the clonal expansion of lymphocytes in response to antigenic stimulation (Chandra, 1990). Chromium is an essential mineral that is thought to be necessary for normal glucose and lipid homeostatsis (Cefalo and Hu, 2004). Some studies have shown that chromium supplement may reduce blood glucose levels in individuals with type-2 diabetes and reduce the need for insulin in those with type-1 diabetes (Fox and Zijad, 1998; Ethan *et al.*, 2007).

Majority of antioxidant enzymes or defense systems of the body and processes involved in lipid metabolism in general make use of mineral elements, and an imbalance in these elements usually leads to nutritional disorders and complications of nutritionally related diseases for example diabetes. The presence of these mineral elements in *Annona muricata* leaves could, therefore, be relevant in exerting anti-hyperglycemic activity and the amelioration of the attendant macrovascular complications. The broad distribution of phytochemicals, minerals and antioxidants in *Annona muricata* leaves studied support, as well as provide a basic rationale for their use in folk medicine. From the foregoing, this work therefore indicates that *Annona muricata* leaves, besides serving as good source of pharmacologically active phytochemicals, antioxidants and thus free radical scavengers may also be useful as supplements in human and animal nutrition as they are biodegradable, environmentally friendly, cost-effective and would meet the demand of Nigeria as a country to go green.

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